Medical Library


# THE AMERICAN JOURNAL OF PHYSIOLOGY

EDITED FOR

THE AMERICAN PHYSIOLOGICAL SOCIETY


## CONTENTS


THE ENDING OF THE AXON ACTION POTENTIAL, AND ITS RELATION TO OTHER EVENTS IN NERVE ACTIVITY. *Herbert S. Gasser and Joseph Erlanger* ..... 247

HEMOLYSIS OF THE ERYTHROCYTES OF AMPHIBIA. *Harry A. Charipper* ..... 278

ON THE PRESENCE OF CHOLECYSTOKININ IN THE HUMAN AND RABBIT INTESTINE. *G. E. Dreweyer and A. C. Ivy* ..... 285

PHYSIOLOGICAL VARIATIONS IN THE CARDIAC OUTPUT OF MAN. IX. THE EFFECT OF BREATHING CARBON DIOXIDE AND OF VOLUNTARY FORCED VENTILATION ON THE CARDIAC OUTPUT OF MAN. *Arthur Grollman* ..... 287

THE REGULATION OF RESPIRATION. A STUDY OF THE CORRELATION OF NUMEROUS FACTORS OF RESPIRATORY CONTROL DURING ALTERED PRESSURES OF ALVEOLAR OXYGEN. *Robert Gesell, Hugo Krueger, Grace Gorham and Theodore Bernthal* ..... 300

THE REGULATION OF RESPIRATION. A STUDY OF THE CORRELATION OF NUMEROUS FACTORS OF RESPIRATORY CONTROL DURING INTRAVENOUS INJECTION OF SODIUM CYANIDE AND RECOVERY. *Robert Gesell, Hugo Krueger, Grace Gorham and Theodore Bernthal* ..... 339

THE REGULATION OF RESPIRATION. A STUDY OF THE CORRELATION OF NUMEROUS FACTORS OF RESPIRATORY CONTROL DURING HEMORRHAGE AND REINJECTION. *Robert Gesell, Hugo Krueger, Grace Gorham and Theodore Bernthal* ..... 365

THE REGULATION OF RESPIRATION. A STUDY OF THE CORRELATION OF NUMEROUS FACTORS OF RESPIRATORY CONTROL FOLLOWING INTRAVENOUS INJECTION OF SODIUM BICARBONATE. *Robert Gesell, Hugo Krueger, Grace Gorham and Theodore Bernthal* .. 387

THE REGULATION OF RESPIRATION. A STUDY OF THE CORRELATION OF NUMEROUS FACTORS OF RESPIRATORY CONTROL FOLLOWING ADMINISTRATION OF HYDROCHLORIC ACID, OF CARBON DIOXIDE, AND THE SIMULTANEOUS ADMINISTRATION OF CARBON DIOXIDE AND SODIUM BICARBONATE. *Robert Gesell, Hugo Krueger, Grace Gorham and Theodore Bernthal* ..... 402

THE REGULATION OF RESPIRATION. A STUDY OF THE CORRELATION OF NUMEROUS FACTORS OF RESPIRATORY CONTROL PRODUCED BY INTRAVENOUS INJECTION OF AMMONIUM CHLORIDE, METHYLENE BLUE, STRYCHNINE AND SODIUM SULPHIDE. *Robert Gesell, Hugo Krueger, Grace Gorham and Theodore Bernthal* ..... 427

CONDUCTION IN DIFFERENT PARTS OF THE SMALL INTESTINE. I. DISTANCE TRAVERSED BY A DISTURBANCE AND RATE OF TRAVEL. *Walter C. Alvarez and Kiyoshi Hosoi with the technical assistance of Mildred Foster and E. C. Woolley* ..... 448

OXYGEN UTILIZATION IN THE LEGS OF NORMAL MEN. I. EFFECT OF POSTURE. *M. Florkin, H. T. Edwards and D. B. Dill* ..... 459

OBSERVATIONS ON HOLDING THE BREATH. *Edward C. Schneider* ..... 464

REFLEXES IN THE HIND LIMBS OF CATS AFTER TRANSECTION OF THE SPINAL CORD AT VARIOUS LEVELS. *S. W. Ranson and J. C. Hinsey* ..... 471



VOL. XCIV—No. 2

*Issued August 1, 1930*

BALTIMORE, U. S. A.

1930

Entered as second-class matter, August 18, 1914, at the Post Office at Baltimore, Md., under the Act of March 3, 1879. Acceptance for mailing at special rate of postage provided for in section 1103, Act of October 3, 1917. Authorized on July 5, 1918

*Made in United States of America*

# ARTIFICIAL RESPIRATION and ETHER APPARATUS

***for use with compressed air***

This is an improved form of the apparatus designed by H. F. Pierce, A.B., B.Sc., (Oxon) and described in the Journal of Laboratory and Clinical Medicine, December, 1923.

The apparatus will supply anesthesia, artificial respiration, and tracheal insufflation for any of the ordinary laboratory animals. It is light and compact and is easily carried about. The controls are located convenient to the hand, and ether, air, or respiratory rate may be varied and adjusted simply and easily.

In operation the apparatus is connected with a supply of compressed air at a pressure of 4 to 10 pounds per square inch. No other source of power is required.

**Price $60.00**

***Manufactured by*** **JOSEPH BECKER**

**630 WEST 168TH STREET, NEW YORK**

THE AMERICAN
JOURNAL OF PHYSIOLOGY

VOL. 94 AUGUST 1, 1930 No. 2

# THE ENDING OF THE AXON ACTION POTENTIAL, AND ITS RELATION TO OTHER EVENTS IN NERVE ACTIVITY

HERBERT S. GASSER AND JOSEPH ERLANGER

*From the Departments of Pharmacology and Physiology, Washington University School of Medicine, Saint Louis*

Received for publication March 8, 1930

Du Bois-Reymond, who first discovered the negative variation of the demarcation potential of nerve during activity, also observed that at the end of a tetanus the needle of his galvanometer always returned more or less incompletely to its original position. Little attention was paid to this observation, however, and the center of interest turned rather toward the positive after-effect which was later described by Hering. It was only when the technique of electrical recording had evolved so that the potentials of single impulses could be measured, that the prolongation of the negative potential was noted with any regularity. Gotch and Burch, working with the capillary electrometer, found that the rapid upward movement was followed by a prolonged "tail" which had a much lower E.M.F. and disappeared relatively slowly. The data for their nerve II, when plotted, give a good general picture of the whole process. It lasted $60\sigma$ at 4° to 6°C. Further work with the capillary electrometer was done by Boruttau and Fröhlich; in their experiments they noted that the negative after-effect increased as the nerve deteriorated. The long duration of the action potential first came to our notice in a paper by Forbes and Thatcher. They found that single responses of frog nerve as recorded with the string galvanometer, after amplification through a vacuum tube, had in certain cases a very prolonged duration. After showing that this long duration was not an artifact introduced by the apparatus they concluded that it must have been due to a seasonal variation in the frogs. Their figure for a normal nerve (fig 26, 1A) had a very definite if much smaller prolongation, however; and, when we came to record amplified nerve action potentials with the string galvanometer, tracings very similar to it were obtained. With the development of the Braun tube technique the same prolongation was again apparent and records were prepared and

demonstrated at the XIth International Physiological Congress at Edinburgh, which showed the prolongation of the negativity in both string galvanometer and Braun tube records taken from the same nerve. In the same year Broemser published records made with the string galvanometer without previous amplification. As he describes the response, every curve shows in addition to the main deflection a distinct, relatively slow after-effect of the same sign but of a relatively lesser magnitude.

The subject has recently received an important contribution in a paper by Levin, to which we will refer in more detail later. He found that each impulse in crustacean nerve leaves behind it a particularly marked "retention of current" which, after repeated stimulation, becomes so great that but little back swing of the galvanometer (Downing type) occurs on cessation of stimulation. These facts have been confirmed by Furusawa. In mammalian nerve both negative and positive after-effects have been mentioned by Gerard and Forbes; and Davis, Pascual, and Rice have observed that the after-effect in the femoral nerve of the cat is increased from $25\sigma$ to $50–100\sigma$, when exposed to $CO_2$ (string galvanometer, 23°C.). Most recently the after-effect in frog nerve has been made a special subject of investigation by Amberson and Downing, with the aid of the latter's galvanometer. When a nerve had been fatigued by a period of tetanic stimulation they found that single impulses were followed by periods of after-negativity lasting up to 1 second; then the deflection regularly passed through zero so that the proximal lead became positive to the distal.

The accumulated evidence thus renders it apparent that from now on it will be necessary in discussions of the axon action potential to speak differentially of its two parts; and therefore it is necessary to settle upon a terminology. After considering various possibilities we have decided upon "spike" and "after-potential." The former term was used by Burdon-Sanderson for the probably homologous element in the muscle action potential; and the latter term has the double advantage that it refers only to a potential and that, while it is entirely non-committal as to whether or not this potential is continuous with the spike, it covers the situation satisfactorily if, as is probably the case, the after-potential is separate. Unless qualified the term will be used as meaning a negative after-potential. We considered the adoption of the term after-effect which has been used in this connection but it was rejected because it has also been used for such phenomena as the refractory period.

A number of problems in connection with the after-potential remain unsolved,—for instance its magnitude with respect to that of the spike, and the relation of both its magnitude and duration to the other measurable events occurring during nerve activity.

THE MAGNITUDE OF THE AFTER-POTENTIAL AS COMPARED WITH THE SPIKE. While some of the methods which have been used have been cap-

able of revealing with approximate accuracy the duration of the after-potential, particularly when the latter is prolonged, none of them has given any information as to its height in comparison with the potential developed at the crest of the spike. This is because the spike is treated more or less ballistically by the galvanometer, while the after-potential is to a very considerable extent directly measured. The relationship of the after-potential to the spike potential was accordingly made the first subject of investigation.

METHOD. While ordinary three-panel amplification is sufficient for the demonstration of the after-potential in Braun tube records, the effect can be studied in much greater detail with four-panel amplification. A direct calibration of the apparatus as used in the following experiments showed that one-tenth of a millivolt gives a deflection of 26 mm. when the Braun tube is operated with a driving voltage of 300. The sensitivity is thus brought into the range of a very sensitive galvanometer, 26 cm. per millivolt, which may be compared with the figure of 35 cm. per millivolt given by Amberson and Downing. As this sensitivity is gained with a system that records without lag, the crest height is accorded its full value and is directly comparable to the after-potential recorded under the same conditions. For practical reasons it is often desirable to operate the Braun tube at 400 volts so single spike potentials may be recorded, and then to measure the after-potential without changing the conditions; in this case the sensitivity is cut down 26 per cent, but it is still more than adequate.

Since the spike height, equivalent to the after-potential recorded, would be two to four meters, depending upon the strength of stimulation and therefore the proportion of the nerve fibers brought into activity, the spike potentials were recorded at three panels, with the input shunted. The full three-panel height was then computed and multiplied by the augmenting power of the fourth panel to obtain the height equivalent to that of the after-potential, which was measured with four panels.

Before proceeding further it was necessary to determine the capacity of the system to take care of potentials of the duration involved, that is, to estimate the distortion due to the series capacities. As a preliminary to this determination all the coupling condensers were increased to 4 mf. and all the leaks decreased to one reciprocal megohm. Two questions had to be answered: 1, how much would potentials of the magnitude of the after-potential seem to decrease during the period of their existence, due to the filling of the series condensers; and 2, for the same reason, how much would potentials of the magnitude and duration of spike potentials on cessation depress the base line, and thereby introduce an error into the reading of the duration of the after-potential and finally simulate a positive deflection of nervous origin.

The first question is answered by the first tracing in figure 1. As the

after-potentials are regularly recorded with four-panel amplification, a potential of 0.1 millivolt was applied to the input of the amplifier when operating at this sensitivity. The figure gives the result when the potential lasted 120$\sigma$ If it be closely inspected it can be seen that there is a very slight depression of the base line, showing that the recorded potential had decreased a few per cent. This amount, which offers no serious error

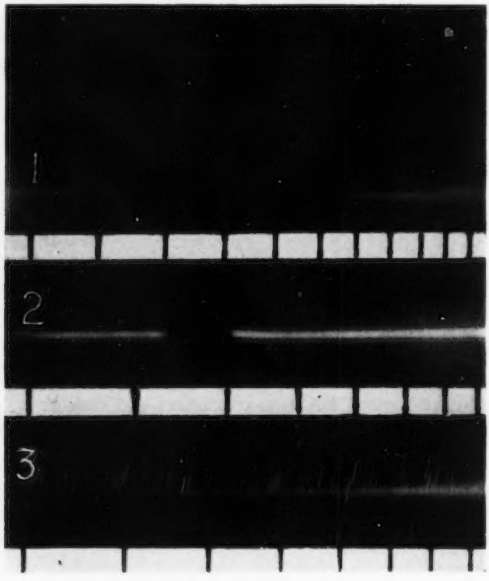



Fig. 1. Calibration of the effect of the series condensers in the recording system; 4 panel amplification.

1. 0.1 mv. input, 0.5 mf., 400,000$^{\omega}$, X = 100. Duration of constant potential 120$\sigma$. Time marked in 25$\sigma$ intervals.

2. 20 mv. input, 0.5 mf., 50,000$^{\omega}$, X = 100. Duration of constant potential 3.75$\sigma$. Time marked in 5$\sigma$ intervals.

3. 30 mv. input, 0.5 mf., 280,000$^{\omega}$, X = 96. Duration of constant potential 8.4$\sigma$. Time marked in 25$\sigma$ intervals.

at 120$\sigma$, would dwindle to insignificance at the durations of the normal after-potentials which are much shorter than this. It would, however, become appreciable in the very long after-potentials found in deteriorated and poisoned nerve, and would to a greater extent tend apparently to shorten the positive phase. The second question is answered by the second and third tracings in figure 1. An input of 20 millivolts was used; this would mean an output potential determined by the limits of the char-

acteristic of the vacuum tubes and would be as great as any ever obtained from a nerve. Tracing 2 shows the beginning and ending of a potential lasting $3.75\sigma$ and makes it apparent that there is no visible discontinuity in the X axis. Tracing 3 is a record made at another time, when the input was 30 millivolts and the duration $8.4\sigma$. Here the filling of the condensers was sufficient to produce a very minute depression of the base line, but the area of the potential-time impressed on the input was at least 15 times that which could arise from any spike potential in nerve. Furthermore, the X axis appears perfectly straight after the cessation of the impressed potential, while the positive after-potential record is curved. Therefore, the positive after-potentials which are to be described cannot be artifacts introduced by the apparatus; and the same may be said for the negative after-potentials, on account of the rectangular ending of the potential-records in figure 1.

The principal source of error in the measurement of the after-potential arises from the fact that the electrical potential at any point on a nerve can only be measured by comparing it with some other point. What is sought experimentally is a comparison of a normal point with one which does not change, that is of a normal portion with a dead portion; but practically this is unattainable, for somewhere there must be a transition from dead to live nerve, and the dead end acts as a lead from the greatly injured nerve at the junction. This gives rise to the diphasic artifact of the spike as has been explained in some detail by Bishop, Erlanger and Gasser. As one goes through the published records of action potentials it is found to be generally present, for instance, in the capillary electrometer records of Gotch and Burch (nerve II), in the string galvanometer records of Forbes and Thatcher and of Broemser, and in our own Braun tube records. When the stimulus is close to the lead the diphasic artifact comes just after the single spike; when conduction is permitted, the portion of it belonging to $\alpha$ and $\beta$ waves often falls between $\beta$ and $\gamma$ giving the latter a fictitious prominence. When high amplification is used and the potential recorded with a slow movement of the spot in the X direction, the diphasic artifact becomes very prominent although the potential of the second phase is so small as to be scarcely visible in three-panel records and amounts to less than one per cent of that of the first phase.

It is impossible to eliminate the diphasic artifact belonging to the spike. Leading close to the killed end causes it to disappear; but it is only moved forward under the crest; the recording of the end of the wave is improved at the expense of some antecedent portion of the wave where the distortion is less apparent. Similarly it is impossible to eliminate the artifact belonging to the after-potential.[1] The amount and duration of the after-potential

[1] This subject will be taken up again in a special section of the paper where Woronzow's admirable analysis of the so-called positive after-potential will be reviewed.

is increased in injured nerve; therefore after a time the after-potential under the live lead is over-balanced by the after-potential at the dead-live junction, and the spot crosses the zero line. We find in confirmation of Amberson and Downing that there is regularly a swing in the positive direction before the resting potentials are restored; and this is still present when the active lead is close to the killed end, though it is then decreased in magnitude, doubtless due to the greater and longer lasting after-potential at the live lead which is less easily counterbalanced. In this sense Hering's contention that the size of the positive after-potential is an index to the condition of the nerve is correct.

The practical aspect of the situation just described is that it is impossible to know how much after-potential exists at the active lead at the time that the deflection passes through zero. The measurements of the after-potentials which we report are made at the point at which the projections of the X axes, as recorded before the deflection starts, intersect the potential curves. They thus represent the minimal durations of the after-potentials; but it will be seen later that in many cases the point selected must be a fairly reliable index as it checks the curve of thresholds of response, which is not dependent on the condition at two parts of the nerve.

The whole procedure involved in getting the relation of the spike to the after-potential may be illustrated by a description of a single experiment which is typical of a series. In this group of observations the attempt was made to obtain an approximately correct picture of the transition from the spike to the after-potential by killing the nerve as close to the active lead as was possible without reducing the magnitude of the response. In the experiment under consideration the spike height was first measured when the killed end was at a distance from the active lead. The nerve was then killed closer and closer to the lead while watching at each step the size of response. At the point chosen the remaining interpolar length of live nerve was so short that it was thought inadvisable to continue; nevertheless a trace of the diphasic artifact was still present, as can be seen by the fact that in figure 2, *2*, the angle between the spike and the after-potential is too acute. A more drastic treatment of the other nerve of the pair from the same frog resulted in complete elimination of the artifact, but the crest height was decreased.

Silver—silver-chloride electrodes were used for both stimulation and leads, after it had been demonstrated that they introduced no error due to polarization. Their adequacy was shown by the fact that the form of the after-potential was the same when calomel half-cells were used as leads, and by the fact that, when a constant potential corresponding in size to that of a nerve, but of much longer duration, was applied to the input at four-panel amplification, in such a way that the silver electrodes, with an intervening stretch of narcotized nerve as a polarizable resistance, were

part of the circuit, the potential was returned in the record in perfect rectangular form. As the axon action potential form was desired the stimulating electrodes were placed close to the leads; in this case 8 mm. intervened between the cathode and the distal side of the proximal lead. The usual method of obtaining the axon action potential is to lead from the stimulating cathode; but this difficult technique, which necessitates the use of root preparations, may be avoided and a good approximation still be obtained if the green frog sciatic be used and a small distance of conduction permitted. If the stimulus be near the threshold the fibers responding are so homogeneous in character that there is no measurable temporal dispersion, and the axon action potential duration is correctly recorded. If, as in this experiment, the stimulus be sufficient to excite approximately the whole $\alpha$ group, the maximal increase calculated from histological data would be $0.1\sigma$, after 8 mm. of conduction. This error is not serious, however, when the values are used only for comparison with those of the after-potential; and the prolongation of the after-potential itself is insignificant (a $0.1\sigma$ addition to values of $20\sigma$ and upward).

All the records in this research were made by single sweeps of the spot across the face of the tube. The spike potential was first recorded with three-panel amplification and with a shunt placed across the electrodes (fig. 2, *1*). From this tracing the height corresponding to the scale of the after-potential records could be calculated by making use of the resistance of the nerve, the magnitude of the shunt, and the calibrations of the amplifier for three and four panels (fig. 2, *5* and *6*). The multiplier was 78.4. Then the after-potential was recorded at four-panel amplification, making use of various speeds so as to obtain optimal data on all its parts (two examples are given in tracings *2* and *3*, fig. 2). From the whole series the graph was plotted.

A final control was made to see if the stimulus artifact produced any distortion of the after-potential. In the first place it can been seen that the artifact has nearly disappeared before the spike begins (tracing *1*) and that therefore distortion of the after-potential is improbable; but to eliminate all possibility of such an event the nerve was stimulated after narcosis with ether. This caused all of the action potential to disappear (fig. 2, *4*), and left as the only visible disturbance in the line a gap at the time of the shock. It may be argued with some justice that the artifact in a narcotized nerve may be different from normal; but, since narcosis is believed to occur with a tightening of the membranes, the expectancy would be that the artifact would increase. In point of fact, observation of the process of narcosis of active nerves reveals that the after-potential decreases when the height of the spike falls and completely disappears when narcosis is complete. The after-potential therefore cannot be brought into relation with the exciting shock, and must be due to the fact that the nerve has been active.

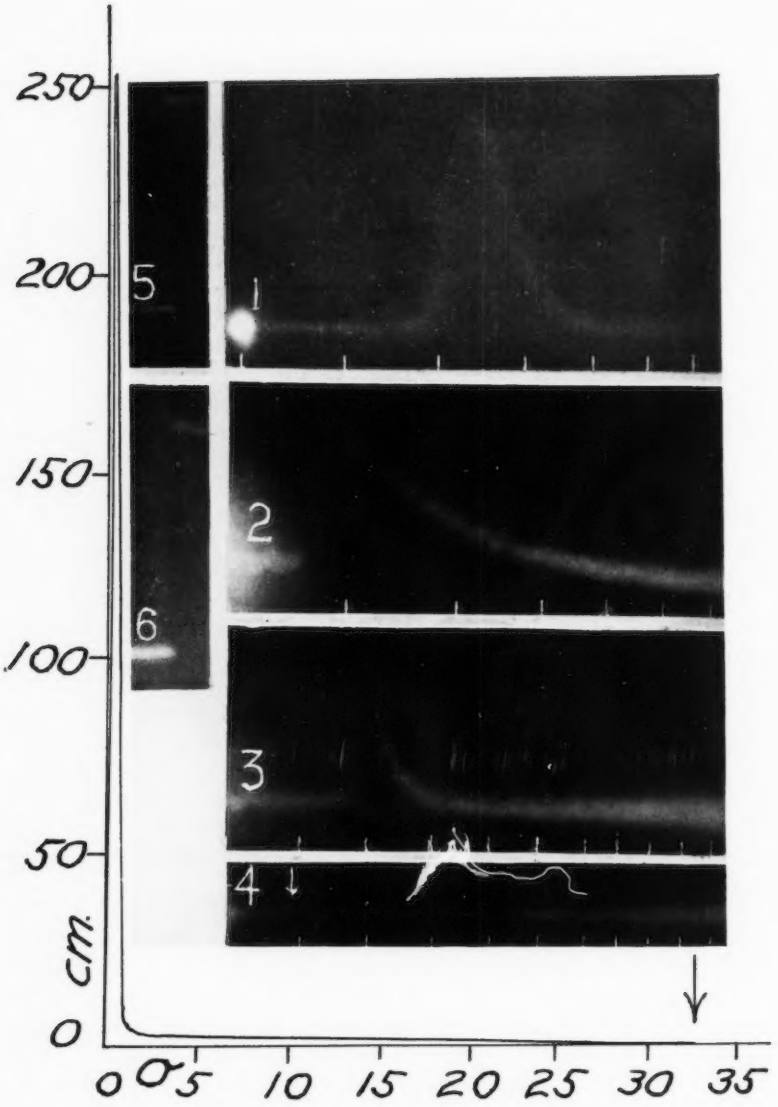



Fig. 2. Relation of spike to after-potential. Insets, records from which the data were obtained.

1. Form of spike potential. 3 panel amplification, nerve shunted with 30,100$^{\omega}$, 0.5 mf., 6000$^{\omega}$, X = 108. Time marked in 0.5$\sigma$ intervals.

2. After-potential. 4 panel amplification, 100,000$^{\omega}$. Time in 10$\sigma$ intervals.

3. After-potential. 410,600$^{\omega}$, time in 25$\sigma$ intervals.

4. Like 3 except that the nerve had been narcotized. The arrow shows position of shock.

5. Calibration at 3 panels, 400 volt anode-cathode P.D. on Braun tube. Input, 4 millivolts.

6. Calibration at 4 panels. Input, 0.2 millivolts.

Except where specifically indicated all the experiments were performed on the sciatic nerve of the green frog (R. pipiens).

Results. The small size of the after-potential as compared with the crest height strikes one at once; in fact the dimensions of the whole process are such that graphic representation is difficult, the two parts appearing as very narrow bands near the axes (fig. 2). When the after-potential is maximal, at $1.6\sigma$, it is only one per cent of the crest height, and thereafter it falls off gradually, to cross the X axis finally at $33\sigma$, at the point marked by an arrow. The negative potential is followed by a very small positive one which ends somewhat after $100\sigma$. This can be seen in figure 2, *3*; but

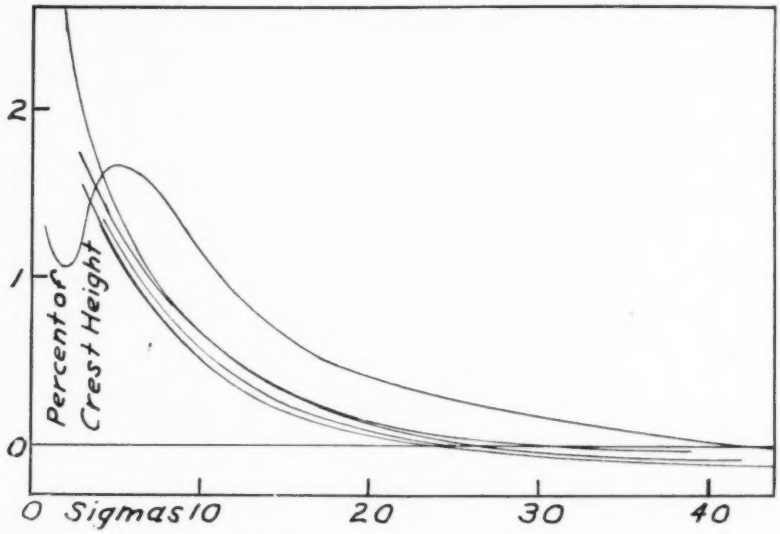



Fig. 3. After-potentials in moderately fresh nerves. Data from five different nerves.

better examples are to be found in later figures. Whether or not the after-potential in this nerve was increased by the proximity of the dead end to the lead cannot be stated; in any case an increase has not occurred to any marked degree, as the duration compared very well with those which hold for nerves which have been recently prepared and subjected to only a short period of experimentation.

In all further work no attempt was made to avoid the diphasic artifact of the spike, and the lead was always made at a safe distance from the killed end. A cross-section of the magnitudes and durations of the after-potential for moderately fresh nerves, as obtained in these experiments, may be

seen in the series of examples selected for figure 3. In four of the five cases the magnitude of the after-potentials cannot be measured in the period at the end of the spike on account of the diphasic artifact of the latter, which enters into the curve in the manner traced out in one of them. But the curves do show that at $4\sigma$ the potential regularly has values of less than 2 per cent of the maximum potential attained during activity, and that the base line is crossed between 20 and $40\sigma$.

As fatigue is known to increase the duration of the after-potential, a number of nerves were observed in which the response to the first break shock was obtained. For this purpose the settings of the apparatus were made on one of a pair of nerves, then the second nerve was mounted and the first response recorded. Following this the necessary data on the

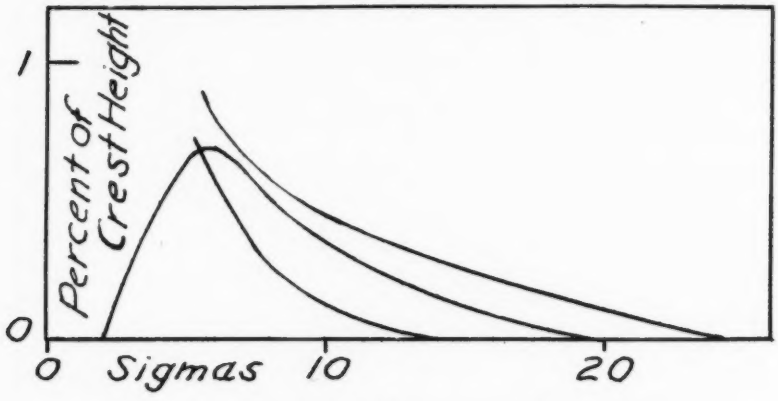



Fig. 4. After-potentials in the first responses of isolated nerves.

height of the spike were obtained, a procedure which is possible because the spike is relatively very much less susceptible to alteration by fatigue. The data obtained (fig. 4), which represent the closest approach to normal which can be attained in an isolated frog nerve, show that when the after-potential can first be measured, that is, after the diphasic artifact of the spike is over, it is less than one per cent of the crest height and lasts $20\sigma$ and less. This duration rapidly increases on repeated stimulation and is also much longer in fresh nerves from poor animals. There is considerable variation from frog to frog, and when the after-potential is initially long it is also easily augmented by moderate activity during experimentation; thus the behavior of the after-potential is the most delicate index to the condition of the nerve.

THE EFFECT OF TEMPERATURE. The season of the year is an important

factor determining the magnitude and duration of the after-potential at laboratory temperature. We feel that Forbes and Thatcher were quite correct in attributing to seasonal variation the long potentials which they obtained; in fact, we observed that with the advent of cold weather the after-potentials were prolonged from the values we have cited to values of several hundred sigmas, although the frogs were selected from the same lot which had served for the earlier part of the series. The duration was much reduced if the experiment was performed at a temperature nearer to that of the aquarium; and if the winter frogs were kept for a week at laboratory temperatures the summer values were again restored.

As is well known, the spike potential is prolonged by cold, but in contrast to this the above observations indicate that the after-potential may be shortened. This is an interesting relationship, and therefore experiments were instituted in order to examine it further. These experiments involved a number of variations of technique which need not be described here. Suffice it to say that when nerve potentials at two temperatures are compared it is necessary to bear in mind that there is a rise in resistance of the nerve itself as it cooled, amounting to about 2 per cent per degree centigrade. This fact would cause no error in the recorded potential if the recording devices were strictly potentiometric, and there is no significant error when the nerve is connected directly to the amplifier input; but a very considerable error is involved if input shunts are employed, and the same is of course true for all galvanometer readings. The cold potentials are recorded at lower than their real values and must be corrected for the resistance change.

In the illustrative case which is presented in figure 5, the procedure was as follows. The spike heights were such that on three-panel amplification without input-shunting the crests of the waves were visible on the screen of the tube. The heights were therefore measured and, when corrected according to the characteristic curve of the amplifier and Braun tube assembly, they had a value corresponding to the portion of the characteristic on which the after-potential was recorded. To get the spike form the input was shunted until the figure came well on the linear portion of the characteristic; then, in transcribing, all the ordinates were multiplied by a factor which would give the crest its real value. The after-potentials were recorded with three-panels unshunted so that their magnitude could be determined; and these records were checked against shunted four-panel records which gave more accurate readings of the form and duration.

Because of the higher threshold of a cooled nerve to induction shocks, precautions are necessary to assure that the same number of fibers are stimulated at the two temperatures. Fortunately this factor is controllable on account of the marked difference between the most irritable of the *B* fibers and the least irritable of the *A* fibers (Erlanger and Gasser, 1930).

Shocks just under the value adequate for $B$ at the higher temperature are so supermaximal for $A$ that there is no danger of any $A$ fibers dropping out as the threshold rises. On the other hand this precaution, necessary to make the potential magnitudes comparable at two temperatures, has one disadvantage; it causes a greater difference between the recorded potential form and the axon action potential form. As has been explained, the axon potential form in this series of experiments is obtained by using short distances of conduction and a homogeneous group of the most irritable fibers. When the whole $A$ group is used, there is some separation of the $\gamma$ potentials and some prolongation of the wave at the base, the principal effect being to fill in the notch corresponding to the diphasic artifact of the faster $A$ fibers. The same factor tends to produce a still greater prolonga-

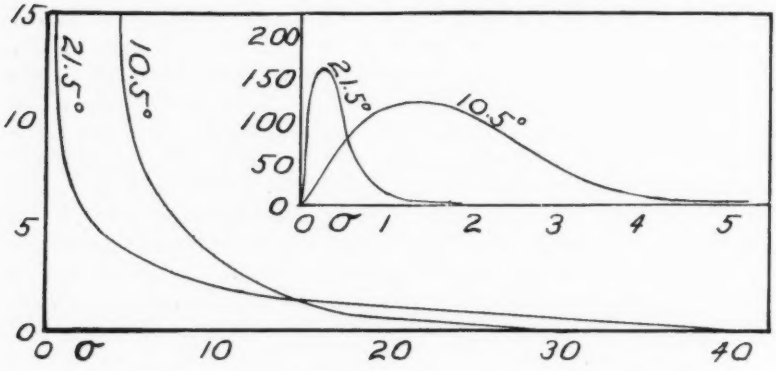



Fig. 5. Effect of temperature upon the after-potential. Abscissae, time in sigmas. Ordinates, height in millimeters. *Inset.* Dimensions of the spike as recorded after 7 mm. of conduction.

tion of the spike in cooled nerve because, even with the same temperature coefficient for all fibers, the absolute temporal dispersion is greater. As so often happens in nerve experimentation one has to seek a condition which is optimum in the most respects.

A typical experiment is cited in connection with figure 5, in which the action potentials at two temperatures are plotted in superposition. The spike of the warm nerve is initially somewhat higher; but owing to the prolongation of the spike of the cold nerve the potential curve of the latter soon comes to lie above that of the former, and it maintains that relationship throughout the portion which is plotted in the inset. In the ensuing period, however, the curves again cross; and a normal potential is restored more rapidly in the cold nerve. There is considerable quantitative variation from experiment to experiment, but all the observations are in accord

in showing that cold decreases the magnitude of the after-potential and usually shortens it materially (see also figs. 7 and 14). This fact, taken in connection with the prolongation of the spike, which has been demonstrated under more rigid conditions than those imposed in these experiments (Gasser, 1928), has a significance which must be considered in interpreting the nature of the potential occurring at the end of activity.

*The positive artifact.* The phase of positivity which succeeds the negative after-potential is regularly much longer than the latter. Its duration in moderately fresh nerve can be estimated by reference to the instances given in figure 6. In the first case the positive phase lasts about $200\sigma$ after a $20\sigma$ negative one, and its value is 10 microvolts, or 0.14 per cent of the crest

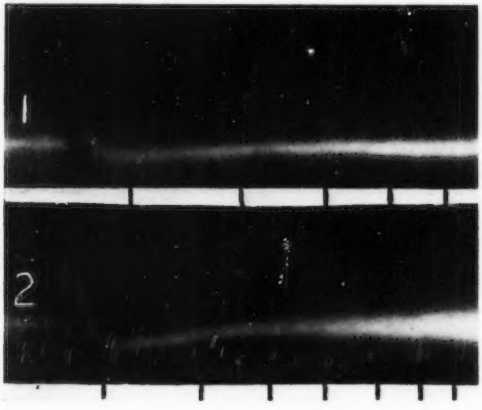



Fig. 6. The positive artifact.

1. Braun tube operated at 400 volts. 0.5 mf., 920,000$^{\omega}$, X = 105. Time intervals marked in 0.1 second.

2. Braun tube operated at 300 volts. 920,000$^{\omega}$, X = 95. Time, 0.1 second.

height; in the second case the positive phase lasts about $260\sigma$ after a negativity of $30\sigma$ and its value is 12 microvolts, or 0.08 per cent of the crest height (the spike in this record on the scale of the portion of the response recorded would be 3.7 meters high). The shortest positive phase which has been measured lasted 125 to $150\sigma$, and the longest were well beyond the durations which can be measured with our apparatus. In general, long positive phases were found to be associated with long negative ones.

Garten observed that the positive after-potential disappears completely when the nerve is cooled down to 8 to 10°C.; and on repeating this experiment we find that the effect is indeed greatly decreased in magnitude, very much shortened, and may, depending upon the interpretation, disappear altogether. In no case, however, does the potential fail to swing to the

positive side. As the positive phase shortens the spike lengthens and thus the diphasic (spike) artifact (fig. 7, lower curve *2*) becomes indistinguishable from the positive after-potential. If the deflection be attributed entirely to the spike, as there is reason for doing, the positive after-potential has entirely disappeared; but then so also has the negative. There is nothing about the positive phase to indicate it to be anything but an artifact; it is another of the unavoidable artificial by-products arising from the necessary double lead from a tissue.

With regard to the older experiments, both Gotch and Cremer expressed the view that the positive after-potentials observed could be explained

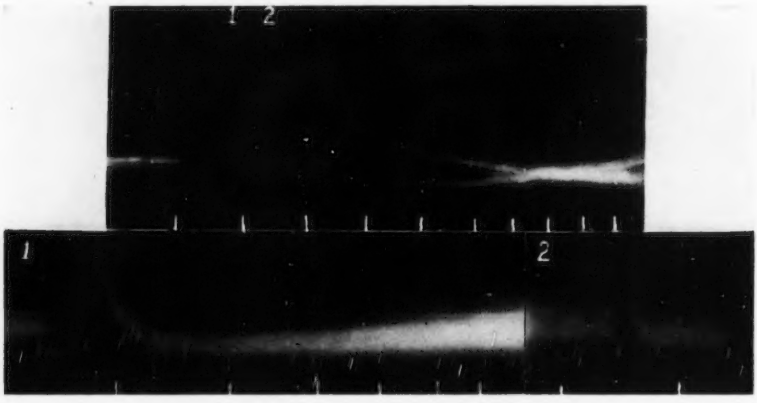



Fig. 7. Effect of temperature on the positive artifact.

*Upper record:* 0.5 mf., 200,000$^{\omega}$, X = 120. Time marked in 10 sigma intervals. Print made through two records superimposed. 1, 23°C. 2, 10.5°. The positive potential has ended in the cold nerve at a time at which it has not yet reached its maximum in the warm one.

*Lower records* made from another nerve. 0.5 mf., 920,000$^{\omega}$, X = 107. Time marked in 100 sigma intervals. 1, 20.5°. 2, 11.5°. The record at the warmer temperature was taken last. Before cooling, the positive swing was shorter than shown in the present record, but much longer than in the cold nerve.

by negativity at the distal lead; and recently Woronzow has made a clear analysis of the manner in which the transition from live to dead nerve brings about such a potential. He established two points; first, that the primary after-potential is negative; and second, that the positive after-potential is due to a temporary increase of the demarcation potential ("Verstärkung des Querschnittes"). The latter fact he proved by producing mechanically a fresh section of the nerve in the course of a tetanus, a procedure which caused the demarcation potential to increase immediately, and the positive swing at the end of stimulation to be very greatly

reduced. In order to bring these facts into line with our treatment of the diphasic artifact of the spike Woronzow's explanation may be paraphrased as follows. When the activity at the dead-live junction causes a negativity which is greater than that at the intact side, a swing of the recording device in the positive direction occurs. This relationship is brought about by the fact that the nerve at the junction is in a state resembling that of fatigued or injured nerve and would therefore have an after-negativity outlasting the change at the intact side. It may be imitated in a diphasic

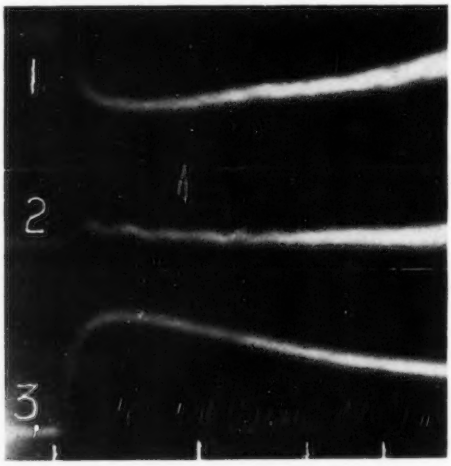



Fig. 8. The after-potential, as seen in a diphasic lead, when the nerve under the leads is differentially poisoned with veratrin; 0.5 mf., 400,000$^{\omega}$, X = 108, 4 panel amplification. Time, 50$\sigma$ intervals. Distance of conduction, 8 mm. Interpolar distance 12 mm. Distal lead 10 mm. from end of nerve.

1. After applying veratrin to the distal lead only. One of a pair of nerves was used for this observation; the normal record of the nerve very closely resembled 2.
2. Normal record corresponding to 3 (other nerve of pair).
3. After applying veratrin to the proximal lead only.

lead by poisoning the nerve under the distal lead only. Such an experiment was performed by Woronzow with cocaine, and we have performed a similar experiment with veratrin, an alkaloid shown originally by Garten and more recently by H. T. Graham to produce a great augmentation and prolongation of the after-negativity. As seen in figure 8, poisoning of the nerve at the proximal lead caused a marked after-potential in the negative direction, while the same condition produced at the distal lead caused the deflection to be in the positive direction. Where activity of a normal nerve is recorded monophasically the lead from the transition zone is less

effective than the one from the side of the nerve, therefore the positive swing is less than the negative phase which precedes it; and when a fresh section is made in the course of a tetanus, as in Woronzow's experiment, the better connection between the inside and outside of the fibers not only increases the demarcation potential, but also lessens the change at the junction due to activity and therefore decreases the positive phase.

The principal support for the view that the positive potential is real is found in Garten's experiments on the effect of temperature. Garten observed that the potential disappears when the live lead is cooled, but that it is unaffected when the cooling is localized at the dead end; and he therefore concluded that the positive-phase is due to an actual positivity at the intact surface. These experiments have rightly been criticized by Woronzow on the ground that it is not the dead end which is in question but the transition zone; and Garten does not indicate that he has considered this distinction. While we have made some observations indicating that cooling the junction does decrease the positive phase, we have dealt mainly with diphasic leads in which the two electrodes would have the same value, and in which, therefore, the effect of cooling the nerve under the distal electrode would produce a more marked effect. In these experiments tubular silver electrodes coated with silver chloride were employed. The temperature of the nerve under the electrodes could be controlled by perfusing the electrodes themselves; and, to prevent the spreading of the temperature effect from one electrode to the other, water at room temperature was perfused through one of the pair while cold water was perfused through the other.

When the electrodes were isothermal, the usual diphasic spike was obtained followed by electrical neutrality, but when the nerve was cooled under one electrode the spike was followed by a potential-picture which can best be understood by comparing it with a theoretical one. The negative after-potential as modified by cold was seen in figures 5 and 7, and although variations occur, depending on the state of the nerve and the temperature, the relationship shown is typical. Accordingly, the theoretical picture was constructed by drawing two monophasic endings in agreement with the types shown in the figures cited, but arbitrary in form. These endings, which are shown in figure 9A, were then combined in the manner in which they would enter into a diphasic wave. A study of the figure reveals the fact that, when the two parts are so combined, the dominant negative potential at the ending of the spike is the one belonging to the cooled phase; therefore, in the construction made for proximal cooling, the initial after-potential is in the direction of the first phase of the spike; but the longer lasting distal negativity soon is revealed as a deflection in the opposite direction (9 B). On the other hand when the cooling is distal the second phase of the spike is directly continuous with its after-

potential and the potential picture is ended by the longer lasting negativity at the proximal lead (9 C).

An actual experiment is also shown in figure 9. On proximal cooling the result was initially as predicted (1), but as cooling proceeded the nerve

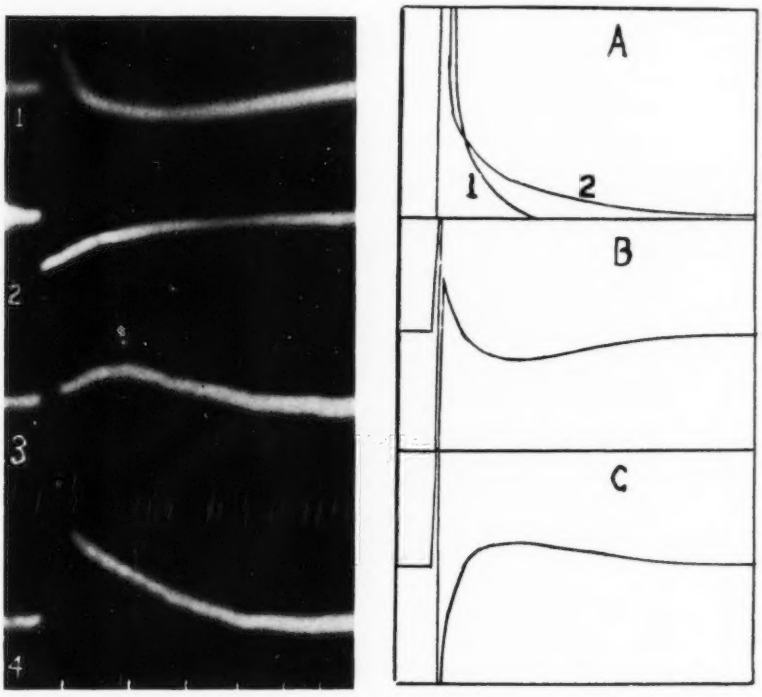



Fig. 9. Differential cooling of nerve under diphasic leads, showing imitation of positive artifact; 4 panel amplification; ordinate, 1 mm. = 4$\mu$v., 0.5 mf., 920,000$^{\omega}$, X = 105. Time marked in 5$\mathcal{C}\sigma$ intervals. Stimulus 9 mm. from proximal lead, interpolar distance 12 mm., distal lead 15 mm. from end of nerve. Shocks maximal for the *A* group of fibers.

*Photographs.* 1 and 2, two stages in the effect of cooling the nerve under the proximal lead; 3 and 4, cooling under the distal lead.

*Diagrams.* *A*. Typical ending of action potential waves as recorded monophasically at two temperatures; 1, cold; 2, warm. B. Diphasic potential which would theoretically be produced when the second phase corresponds to the potential of the cold nerve. C. Ditto, when the first phase has the potential of the cold nerve.

at the cooled end became so depressed that the potential there was overpowered, with the result that the curve took on the appearance of a monophasic lead from the *distal* electrode (2). Similarly on distal cooling there

was at first some evidence of the distal after-potential (3), but later the picture was converted into one resembling that obtained from a normal monophasic lead (4). This experiment can have but one interpretation: *the positive effect at the proximal lead is due to negativity at the distal*,—it has the same duration as the negative after-potential recorded monophasically from the same nerve at the warmer temperature. The positive after-potential in a monophasic lead follows the negative, and no counterpart for it is seen in intact nerve.

Our experiments on differential cooling are directly opposed to those of Garten both as to findings and to conclusion. When Garten led diphasic-

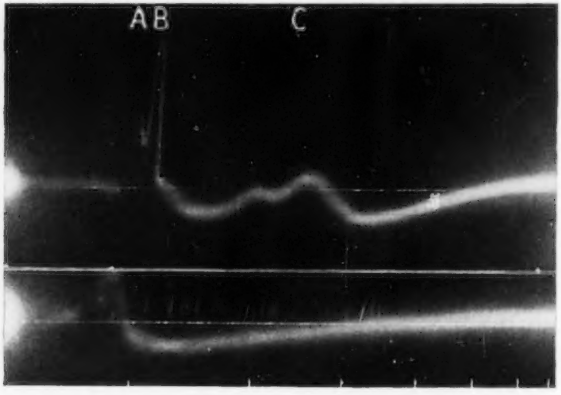



Fig. 10. After-potentials when all the fibers in a nerve are stimulated. Ordinate, 1 mm. = 4$\mu$v., 0.5 mf., 920,000$^{\omega}$, X = 167. Time marked in 100$\sigma$ intervals. Bullfrog sciatic; conduction distance 100 mm.

*Upper curve.* Spikes, after-potentials and artifacts when all the fibers in the nerve are stimulated. *B* wave retouched.

*Lower curve.* Shows the positive artifact when the *A* and *B* fibers but not the *C* fibers are stimulated.

ally and recorded with a string galvanometer he found that at the cessation of a tetanus the after-swing was always in the direction of the phase corresponding to the cooled end, which fact he interpreted as showing that a real positive potential was revealed at the warm end of the nerve when its counterpart at the cooled end was depressed. To attempt to reconcile Garten's experiments with our own is hazardous. If the condition following a tetanus resemble that following a twitch, and if the experiments be reconcilable at all, then it is necessary to assume that Garten's after-potentials represent dominant negativity of an unexpected duration at the cooled end; and the only evidence for this is, that in his figure 18, plate XX, made when the cooling was at the proximal lead, the negative after-poten-

tial is followed by a positive. If the condition be different, he is dealing with an event with which we have had no experience.

The same may be said about Verzár's experiments. Verzár found under certain conditions that an extrapolar current, in a nerve polarized with a constant current, is increased in the period following a tetanus. This means an increase of the polarizability of the nerve and he holds it to be identical with the positive after-effect. As it only occurs after prolonged stimulation with strong shocks, the underlying phenomenon is something foreign to our experiments; and it is clear that further work is still necessary to bring out the interpretation which will clarify the whole situation.

Amberson and Downing have described a second negative after-potential following the positive. We have repeatedly looked for this manifestation in fresh summer frogs in which the after-potentials are short; but under conditions which yield the axon action potential form we have never seen it. If the positive after-potential is an artifact, as we believe, then a second negative after-potential becomes highly improbable; it can, however, be imitated if a sufficient distance between the stimulus and leads be allowed and if all the fibers in the nerve be stimulated. Then negative potentials of the late waves, *B* and *C* (Erlanger and Gasser, 1930), appear following the after-potentials of the *A* wave. In fact, a whole series of phases occurs belonging to the spikes, after-potentials and artifacts due to each of the three constituents (fig. 10). The *B* wave appears on the after-potential of *A*, and at its base can be seen a small negative wave due to its diphasic artifact. Following the after-potentials of the first two components, the positive artifact occurs; then the *C* spike appears and the potential difference is in the negative direction again. The diphasic artifacts of late waves are much greater than those of the *A* wave, and that of the *C* wave again causes a big swing in the positive direction. The magnitude of the latter may be estimated by comparing the positive potential with that remaining from the *A* and *B* waves as revealed in the record below it. Then, as the record ends, the potential is negative again due to the after-potential of the *C* fibers which is relatively large. In a lead from the same nerve at 4.8 cm. of conduction, the *C* after-potential appeared more definitely marked. Theoretically there should also be a final positive artifact of the *C* wave, visible to instruments sufficiently sensitive and capable of following the potential for a sufficient period.

RECOVERY FROM EXCITATION AND THE AFTER-POTENTIAL. The two events known to occur following the wave of activity are the refractory period and the supernormal phase.

*The refractory period* will be considered first. The end of the absolutely refractory period was located by Adrian (1921) near the base of the spike, and this location has been confirmed on numerous occasions in this laboratory under conditions which would permit the recording of the axon action

potential form, as opposed to the composite picture of activity in the nerve as a whole. The return of responsiveness, beginning as it does near the beginning of the after-potential, immediately suggests the necessity of comparing the relatively refractory phase with the latter. In mapping the relatively refractory period two features of recovery must be distinguished: first, the threshold of response and, second, the magnitude of the response when stimulation occurs. The first is the feature usually measured and it gives information as to the irritability of the most irritable fibers at various intervals after the start of activity. The second feature, the size of the response, provided the stimulus is maximal, is one which is recognized at times (Davis, 1926), but one which is generally not sharply differentiated from the first. Actually the curves obtained by the two methods are different.

In our technique three procedures have been followed. In all of these the rotator, which synchronizes the stimulation and the deflection of the spot of the Braun tube along the abscissa, has been used to open in succession two break keys in the primary circuits of two induction coils. Harvard coils with their cores removed were employed; and, in order to prevent complication by any possible local effects, the nerve was stimulated at two points, the first stimulus being applied about 2 cm. from the lead, and the second stimulus at a distance of 7 or 8 mm., that is, at a great enough distance to avoid a serious shock artifact but close enough to permit the obtaining of the axon action potential form as explained in the introduction. The constant rate of the rotator made it possible to obtain the desired intervals of stimulation by adjustment of the carriers of the keys which operate on a rack and pinion. The rotation rate being once in four seconds, it was possible to select single pairs of stimuli for recording; therefore the nerve was not fatigued by continuous stimulation. The interval between the stimuli was measured on the Braun tube and a correction made for the conduction time between the two pairs of electrodes. Usually it was sufficient simply to measure the time between the responses, a method which was possible because the proximity of the second stimulus to the lead would eliminate any serious error due to the slower conduction of the second response in all cases except where the responses were very close to the absolutely refractory phase. As the main interest lay in the ending rather than the beginning of the relatively refractory phase no attempt was made to obtain readings very close to the period of absolute refractoriness. The data were obtained by photographing with duplitized x-ray films single pairs of responses.

The first procedure was to determine thresholds, the strength of the shock being measured by the resistance in the primary circuit. As described by Adrian and Lucas (1912), the threshold not only returns to normal but falls below normal: thus the end of the relatively refractory

phase is also the beginning of the supernormal phase. This point as well as the whole course of the supernormal phase may be determined by a much easier and quicker method, as follows.

The second method was one in which the first shock was chosen so as to give an approximately maximal spike; the second shock was then made definitely weaker than the first. With this arrangement the second response was subnormal until all the fibers, normally stimulated by the second shock, were responding and were yielding a maximal potential. That point marked the end of the relatively refractory period. Then, because the first shock stimulated more fibers than the second and because the thresholds were subnormal in the supernormal phase, the second shock stimulated more than the normal number of fibers as soon as it was separated from the first shock by an interval longer than the relatively refractory phase. The height of the second response was thereby made supernormal, and the whole course of the supernormal phase could be followed, since the greater the supernormality the larger the extra number of fibers stimulated and the higher the spike. In this way the duration of the supernormal phase is given exactly; the degree of supernormality is not revealed directly by the height of the spike, but the form of the curve obtained sufficiently resembles that obtained by the threshold method to justify the procedure. The data can be readily obtained by photographing in rapid succession the pairs of responses at different intervals; and, since the supernormal phase is very easily changed by fatigue, the method has the great technical advantage of involving a minimum of stimulation. A more rapid variation of this method is to plot on the screen of the Braun tube the heights of the second responses at the various intervals.

Since in the second method all the $\alpha$ and most of the $\beta$ fibers are in activity, the method can only be possible if the relatively refractory periods for the $\alpha$ and $\beta$ fibers are identical. It has been shown by Erlanger, Gasser and Bishop that the absolutely refractory periods are the same for $\alpha$, $\beta$, and $\gamma$ fibers, and this fact has been repeatedly confirmed in unpublished observations made under better experimental conditions than those holding for the data published. We have now shown that for at least the $\alpha$ and $\beta$ fibers the relatively refractory periods are, within the limits of accuracy attempted, also the same. This was done by comparing the sciatic and skin nerves of the same bullfrog by the first method. Since the most irritable fibers of the skin nerve are $\beta$ fibers, the latter can be directly compared with the most irritable of the $\alpha$ fibers in the sciatic. The result is shown in figure 11; the curves are alike, and such deviations as are present are not constant from nerve to nerve. A comparison of the supernormal phases in the two fibers is not practical on account of the great variation in this feature in one and the same nerve.

The third procedure was designed to determine the return of the ability

to respond. Such a curve can be obtained if the two shocks are maximal at all times; but as the chief point of interest is the time at which the magnitude of the second response returns to normal, it is possible to avoid the strong shocks and still find this point if one merely make the second shock considerably stronger than the first. The fibers stimulated by the second shock and not by the first then contribute a constant height to all the spikes, and one measures on top of this height the magnitude of the responses in the fibers stimulated twice. The relatively refractory period ends when the second response reaches normal height.

The curves of recovery as determined upon one nerve by the three methods can be seen in figure 12. First, the threshold shocks necessary for the second response were determined; then the heights of the second

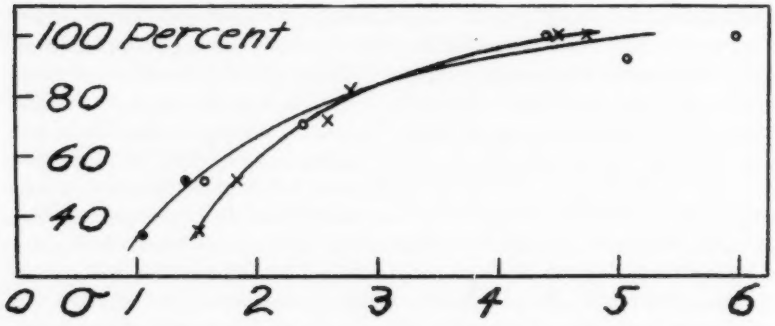



Fig. 11. Irritability of nerve during the relatively refractory period, as measured by the primary current giving a threshold response. Ordinates, percentage of normal irritability. Abscissae, time in sigmas.

○○○○ Skin nerve of the bullfrog ($\beta$ fibers).

×××× Sciatic nerve of the same frog ($\alpha$ fibers).

response, as measured when the second of the two stimuli was considerably stronger than the first; and finally, the heights as measured when the first shock was the stronger. In the first instance the results are plotted as percentage irritability and in the last two the heights are plotted as percentages of those evoked by the shocks used for the second stimulus when acting on the fully recovered nerves. It is seen that the three curves intersect at the ending of the relatively refractory period; therefore the latter can be determined by any one of the three methods. Several other points are also illustrated by this experiment. 1. The supernormal phase is purely a phenomenon of thresholds; the ability to respond, that is, the size of the axon action potential, reaches a maximum at the end of the relatively refractory phase. From then on the spikes attain a constant elevation above the resting potential, though they are cut off at the base

by the size of the after-potential at the moment, just as occurs in an exaggerated form in Levin's observations on nerves of the spider crab. 2. The relatively refractory phase must be the same in slower $A$ fibers as in threshold fibers or the curves would not intersect. 3. The number of fibers stimulated by the second shock is very greatly increased when their irritability is supernormal due to previous activity: a shock normally producing a spike about half maximal in height then produces one twice as high and thus stimulates nearly all the $\alpha$ and $\beta$ fibers in the nerve.

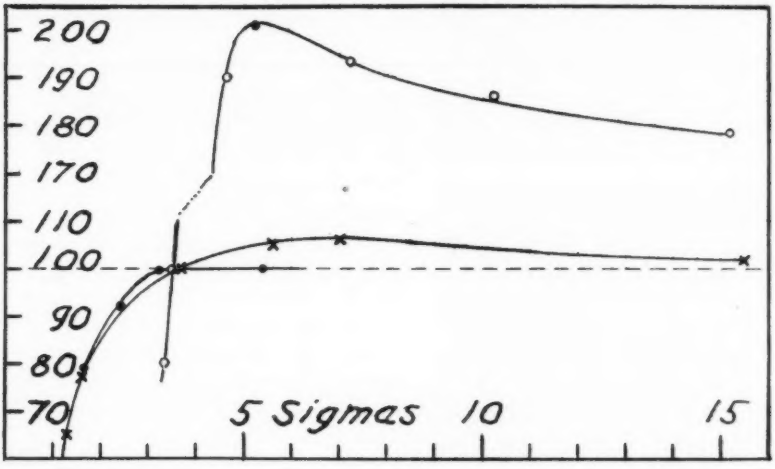



Fig. 12. Recovery of nerve after excitation. Ordinates, per cent of normal.
×××× Irritability determined by threshold of the second response.
○○○○ Height of second response when the first stimulus is stronger than the second.
..... Height of second response when the first stimulus is weaker than the second.

The relatively refractory periods which we have observed at room temperature (20°–24°C.) have fallen in the range from 3.5 to 8$\sigma$, values always smaller than those determined for the end of the after-potential. The shortest after-potentials observed in green frog nerves were 14$\sigma$ long and therefore considerably longer than the refractory phases. This discrepancy is sufficient to discredit any possible relationship, and if any doubt remain it is dispelled by the fact that, when the after-potential is greatly prolonged by fatigue, the two values become still more discordant, as the refractory-period values are changed relatively little. Thus while there is a visible potential throughout the refractory phase there are no events which mark either its beginning or its end. It will be seen later, however,

that in very cold nerve (nerves from summer frogs at 10°C.) the relatively refractory period is greatly prolonged and ends with the action potential.

The duration of the relatively refractory period is here determined as the time at which the height of the spike of the axon action potential returns to normal. The values found are thus very different from those recently determined by Zotterman and by Amberson and Downing. The latter determined the separation of two shocks necessary to cause the Downing galvanometer to double its swing, that is, when working ballistically, to record an area made up of two equal events. Zotterman found that intervals of $50\sigma$ were necessary and Amberson and Downing extended

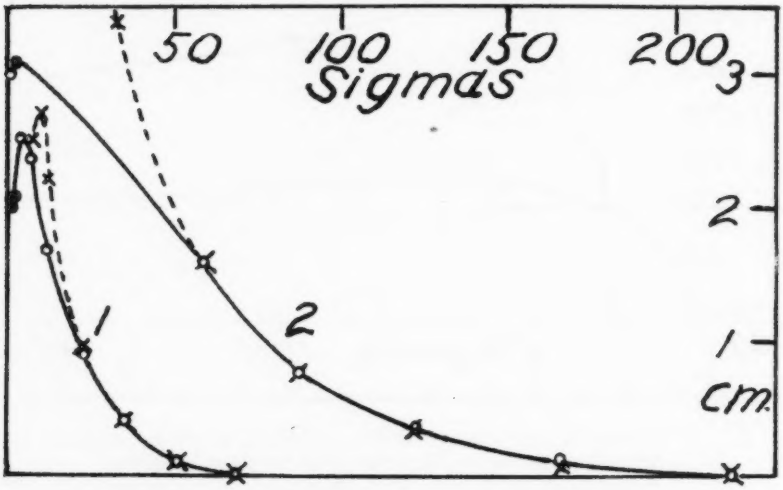



Fig. 13. Showing the temporal correspondence between the after-potential and the supernormal phase in the same nerve under two conditions; 1, when fresh; 2, after a period of experimentation.

○○○○○ After-potentials. ×××× Supernormal phases.

this value to $100\sigma$. These results, as the latter state, take into account the areas involved in the after-potential; ours do not.

*The supernormal phase.* Adrian and Lucas (1912) showed that following the relatively refractory period a nerve may be stimulated by induction shocks too small to excite a resting nerve. This effect they proved to be caused by a change resulting from activity and not to arise from an alteration of the nerve by the stimulating shocks, nor to be due to a change in the nerve's ohmic resistance. Adrian (1920) later mapped the curve characteristic of excitation during the supernormal phase and found that the increased irritability is expressed both by a fall of the rheobase and a shortening of chronaxie.

Just as frog nerves vary greatly as to the magnitude of their after-potentials, they also vary greatly in the degree to which they develop supernormal responsiveness. As the nerves are used routinely in the laboratory a supernormal phase is quite regularly demonstrable; but in very fresh nerves it may be absent or only present in the slower *A* fibers. Ordinarily it increases in intensity, but occasionally a nerve is found which resists its development to an astonishing degree. The Ringer's solution used was not buffered, but occasional tests showed it not to be acid.

When the supernormal phase and the after-potential were compared they were found to be coexisting phenomena. When the after-potential was prolonged by fatigue or the action of drugs the supernormal phase was also prolonged. Usually at the same time the degree of supernormality was greatly increased but this was not without exception. In old preparations, although the after-potentials were longer, the supernormality was at times found to be of low degree although the durations of the two corresponded. Evidently supernormality depends upon some other factor than the after-potential alone.

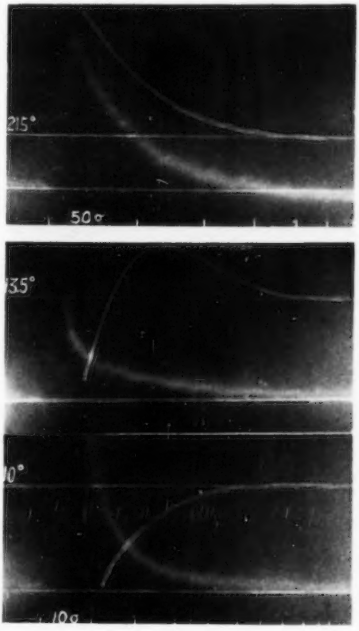



Fig. 14. The effect of temperature upon the supernormal phase; 21.5° record, 0.5 mf., 420,000$^{\omega}$, X = 105; 13.5° and 10° records, 0.5 mf., 150,000$^{\omega}$, X = 105.

Figure 13 shows the after-potentials and supernormal phases in a single nerve under two conditions: 1, when the nerve was fresh; 2, after it had been subjected to considerable experimentation, but was still in excellent condition as judged by the spike-potential. The supernormal phases were recorded by the second method using three-panel amplification. The increase in centimeters of the height of the second response, as affected by various intervals after the first response, is plotted just as it was measured on the face of the tube; and with this is plotted, also in its actual dimensions, the after-potential as measured using four-panel amplification. Therefore only the shape of the curves and not their height is significant; but this is sufficient to show that the two phenomena are very closely related.

The after-potential shortens as a nerve is cooled; and, such being the case, if the after-potential and supernormal phase be associated, the latter should also shorten on cooling. This turned out as predicted; and the results of such an experiment, made on a summer frog, are reproduced in figure 14. The after-potentials and supernormal phases were determined as described in the preceding paragraph. For the latter the same coördinate system on the screen of the Braun tube was maintained as for the former, a procedure which permitted drawing the supernormal phases, as actually mapped on the screen, on the film-impression of the after-potential. Therefore again the form of the curves has significance but not their absolute height. The upper horizontal line is drawn at the height of the isolated spike produced by the shock used to elicit the second response, therefore ordinates above this line mean supernormality. The upper record, taken from a nerve at 21.5°C., shows only the end of the supernormal phase, as the refractory period could not be determined accurately with so slow a spot. The parallelism between the supernormal phase and the after-potential is apparent. When the nerve was cooled to 13.5°C., both of these features shortened together, as a reference to the time intervals reveals. The former pair of curves has about two and one-half times the duration of the latter. Fatigue as a complicating factor was eliminated because the data at the higher temperature were obtained between two identical sets of observations made at the lower. When the nerve was cooled to 10° a most interesting development occurred. The spike which had been prolonged at 13.5° was still further prolonged. At the same time the supernormal phase disappeared altogether and the relatively refractory phase, now having a duration of 60 to 70$\sigma$ lasted as long as the action potential.

*After-potential and supernormal phase in relation to acidity.* In view of Adrian's (1920) careful experiments on the control of the supernormal phase through the hydrogen ion concentration of the solution in which the nerve is bathed, it was necessary to try out the effect of the latter upon the after-potential. As was to be expected the duration and intensity of the after-potential increased when the nerve was transferred to buffered Ringer's solution on the acid side of neutrality, while the change was absent when the transfer was to an alkaline solution. Adrian found that, on the transfer of a nerve from pH 6 to pH 8.5, 10 to 15 minutes' exposure was necessary before the supernormal phase disappeared, and he spoke of this tendency to retain the previous form of recovery curve as a sort of hysteresis. In our experiments we found this persistence to be true to a much greater extent than is described by Adrian (very probably because our nerves were at higher temperatures). Nerves developing a good supernormal phase in acid were found to retain it even when they had been at pH 8.5 for two and one-half hours. They also maintained a well developed

after-potential. As there can be no doubt that all parts of the nerve were in equilibrium with the alkaline bath, it is necessary to conclude that it is not the fact of acidity which is responsible for the supernormal phase, but a change in the nerve produced by acid, a change which can be produced by other means and which can exist in an alkaline nerve.

Discussion. All the evidence at present available indicates that the total electrical change resulting from a single nerve response has two parts. Bishop expressed such a view and it was also clearly seen by Levin, who independently based his opinion on an analysis of Garten's records and a survey of the literature, which brought to light the fact that the spike changes independently of the after-potential. The latter line of evidence has since been augmented by the work of Davis, Pascual and Rice on the effect of carbon dioxide on the action potential of mammalian nerve; and our own observations are in accord with the view. In addition to numerous observations on the lengthening of the after-potential under various conditions in which the spike is unaltered, we have noted that the temperature coefficients of the two events are actually opposite in sign.

The nerve during the after-potential behaves, as far as the investigations have gone, as though it were cathodally polarized, or, in theoretical language, as though the plasma membrane were incompletely reformed. The threshold of excitation is lower, the spike height above the after-potential (but not above the resting potential) is decreased, and the permeability is less. The latter fact was demonstrated by Verzár (1924) in his experiments in which he showed that extra-polar polarization currents are decreased in the period immediately after a tetanus.

Levin conceived the after-potential as being due to the effect of catabolites. In the manner of the liquid chains of Beutner a static potential was supposed to occur until the nerve was restored, a notion which fitted very well with the fact that, as measured by Downing, Gerard and A. V. Hill, 90 per cent of the heat production in nerve takes place during the recovery phase. Later the connection of the after-potential with oxidative recovery was established by Furusawa, who proved that the potential persists when the nerve is deprived of oxygen.

Granted the necessity of oxidative recovery for the restoration of resting potential, there still remains a difficulty in the temperature coefficient. Cooling could produce a decrease of potential, and the probability of its doing so could be ascertained by studies instituted to find whether the temperature coefficient has the right magnitude; but the shortening of the after-potential could not be so explained. If the restoration of the potential mean oxidative recovery, a chemical temperature-coefficient must enter into the calculation, and such a coefficient has the wrong sign. One is driven to make further assumptions.

We can think of nerve activity as being composed of two types of proc-

cesses, one involving the breakdown and reconstitution of the plasma membrane, the other consisting of chemical reactions. There must be an interrelation; but some degree of independent variation may be possible. When an electrical shock is applied to a nerve it is inevitable that ions should accumulate at barriers, as proposed in the theories of excitation. Now is the result of this accumulation first to cause a breakdown of the plasma membrane with a resultant chemical reaction, as is the common supposition; or is the chemical reaction primary? We propose to argue in favor of the latter alternative. The heat experiments of Downing, Gerard and A. V. Hill afford strong evidence of an exothermic chemical reaction in the first phase of nerve activity; and the observation by Gildemeister and by Kato that subminimal stimuli lead to a loss of irritability indicates that a process of some sort takes place before the propagated disturbance is started. If the postulated chemical reaction be sufficiently great then the membrane breaks down, the spike potential is produced, and the nerve becomes completely refractory until the resting potential is nearly restored. Restoration of the surface film seems therefore to be a necessary condition for the return of irritability; but clearly it is not a sufficient one. During the period in which the ability to respond progressively increases and the threshold of response progressively decreases, there is relatively little further restoration; and there are no distinguishing events in the potential curve to mark the period. In fact, except in very cold nerve, the ability to respond becomes normal while there is very definitely a residual negative potential. Another factor is necessary, and it can be provided by making a very simple assumption, namely, that in nerve activity some substance is broken down and then reformed from a precursor. On this basis the ability to respond would return to normal when the reformation is complete, and following this, if the membrane be not also restored, the threshold would be subnormal and the nerve would pass into its supernormal phase.

The foregoing formulation leaves us with undisposed of products of chemical decomposition, the metabolites demanded in Levin's theory. We have also the after-potential, the necessity of oxidation for its removal (Furusawa), and a duration of the after-potential which decreases on cooling. Several solutions of this problem suggest themselves. One possibility is that, although the potential-time area of the spike in cold nerve is larger, the energy cost may be less on account of the slower change. Then if the membrane closes when the reaction products are reduced below an assigned value the closure might occur earlier in spite of the slower reaction velocity. This possibility cannot be pursued further because the energy cost of a single response at two temperatures does not seem to have been investigated. It seems improbable. Another possibility is that the surface film itself is stabilized by cold so that the full potential redevelops

in the presence of a greater concentration of reaction products. This view is compatible with the higher thresholds of excitation of cooled nerve. Finally it may be possible that, although oxidation is necessary for restoration of a very fatigued nerve, restoration to the previous resting condition, as it occurs in a single process, may be taken care of by diffusion. The temperature coefficient would then theoretically be smaller, making it more easily possible for the membrane to be reconstituted in cold nerve.

Another question which demands treatment is how far the course of the spike potential is influenced by a chemical reaction and by a chemical reaction which is at the same time an underlying cause of the refractory state. Although this question can only be handled in a fragmentary way at the present time, the temperature experiments which have been described are highly suggestive. In very cold nerve the restoration of normal potential and the end of the relatively refractory period occur together. May this not mean that there is after all a relation between the spike potential and refractoriness? This question raises another. Where is the transition from spike to after-potential? Although the degree of parallelism has not been determined by simultaneous experiments on single nerves we do know that as the nerve cools the spike prolongs and with it the refractory period, while the after-potential shortens. The supernormal phase also shortens and it finally disappears altogether (fig. 14), which may mean that the after-potential also disappears, and that whatever potential is left all belongs to the spike. In warm nerve then the ending of the spike, as associated with the refractory period, would be obscured by an alteration of the membrane from another cause.

## SUMMARY

The development of the knowledge of the electrical response of nerve in the hands of various observers demands that the action potential be considered as composed of two parts. For reasons stated in the text we have called these parts the "spike" and the "after-potential." The present research deals with their relative magnitudes and their relation to other events attending the nerve impulse.

The potentials have been measured with the aid of the Braun tube oscillograph and amplification giving a sensitivity of 1 mv. = 26 cm.

As previously described, the fast part of the spike of the axon action potential of frog's nerve ends at about $1\sigma$ (20°–24°C.). In the immediately succeeding period, the ordinary "monophasic" lead does not accurately reveal the true potential on account of the diphasic artifact of the spike; but within 1.6 to $4\sigma$ the potential is found to have fallen to between one and two per cent of the maximum. It then declines along a decremental curve and becomes zero at 30 to $50\sigma$, provided the nerve has been subjected to only a moderate amount of experimentation.

When the first response after mounting is measured, the after-potentials end at 14 to 20$\sigma$ (summer frogs at 20°–24°C.); therefore, in nerves of living frogs they would probably be still shorter. On the other hand, in confirmation of other investigators, we have found that the after-potential is greatly prolonged by fatigue.

Nerves from winter frogs as observed at room temperature have long after-potentials.

Cooling a nerve causes a decrease in magnitude and a shortening of the after-potential. The latter fact is in contrast with the lengthening of the spike which occurs under the same condition.

The after-potential is followed by a positive deflection which is explained as an artifact analogous to the diphasic artifact of the spike, that is, as a lead from the dead-live junction through the distal electrode. The experimental basis for this opinion is as follows:

a. Cooling shortens the positive deflection and the after-potential until the former fuses with the diphasic artifact of the spike.

b. The deflection may be imitated in a diphasic lead by increasing the after-potential at the distal lead with veratrin, or by decreasing it at the proximal lead by cooling.

In order to measure the recovery of nerve for comparison with the after-potential, two rapid methods were devised so that the after-potential would not be seriously altered during the measurements. One method reveals the nerve's irritability, the other its ability to respond. The spike height (ability to respond) and the irritability return to normal together at the end of the relatively refractory phase; then the irritability rises above normal, while the spike height (above the resting potential) remains constant. There is thus no supernormal response in the supernormal phase; the thresholds are lower but the spikes are alike except for the small amount of after-potential from the first response which cuts them off at the base.

The relatively refractory periods of the $\alpha$ and $\beta$ waves are alike.

At room temperature neither the beginning nor ending of the refractory period coincides with any definite event in the potential wave; but in very cold nerve the relatively refractory period is greatly prolonged and ends with the negative potential.

The supernormal phase starts at the end of the relatively refractory period and extends throughout the period of the after-potential. The two phenomena vary in duration together.

The after-potential is prolonged and augmented by acid, an observation which fits with Adrian's observations on the supernormal phase. It is the after-potential, however, and not the acidity which is the necessary condition. When the after-potential persists after a long sojourn in an alkaline buffered Ringer's solution, supernormality also persists.

The supernormal phase is shortened by cold in parallel with the shortening of the after-potential and in very cold nerve it disappears altogether. At this time the relatively refractory phase lasts until the end of the action potential, therefore it is reasonable to suppose that the after-potential has disappeared and that all the potential belongs to the greatly prolonged spike.

An attempt is made to harmonize the foregoing observations in a theoretical discussion. It is assumed that while the spike and refractory period are controlled largely by a chemical reaction, the after-potential and supernormality are dependent on the state of the plasma membrane. To explain the shortening of the after-potential by cooling a stabilization of the plasma membrane is proposed.

## BIBLIOGRAPHY


ADRIAN. 1920. Journ. Physiol., liv, 1.
1921. Journ. Physiol., lv, 193.
ADRIAN AND LUCAS. 1912. Journ. Physiol., xliv, 68.
AMBERSON AND DOWNING. 1929. Journ. Physiol., lxviii, 1, 19.
BEUTNER. 1920. Die Entstehung elektrischer Ströme in lebenden Geweben. Stuttgart.
BISHOP. 1927. This Journal, lxxxii, 462.
BISHOP, ERLANGER AND GASSER. 1926. This Journal, lxxviii, 592.
BORUTTAU AND FRÖHLICH. 1904. Pflüger's Arch., cv, 444.
BROEMSER. 1923. Zeitschr. f. Biol., lxxviii, 139.
BURDON-SANDERSON. 1895. Journ. Physiol., xviii, 117.
CREMER. 1909. Nagel's Handb. d. Physiol., Braunschweig, 903.
DAVIS. 1926. Physiol. Reviews, vi, 547.
DAVIS, PASCUAL AND RICE. 1928. This Journal, lxxvi, 706.
DOWNING, GERARD AND HILL. 1927. Proc. Roy. Soc. B, 100, 223. See also, GERARD 1927. Journ. Physiol., lxii, 349.
DUBOIS-REYMOND. 1849. Thierische Elektricität. Berlin, ii, 565.
ERLANGER AND GASSER. 1930. This Journal, xcii, 43.
FORBES AND THATCHER. 1920. This Journal, lii, 409.
FURUSAWA. 1929. Journ. Physiol., lxvii, 325.
GARTEN. 1910. Pflüger's Arch., cxxxvi, 545.
GASSER. 1928. This Journal, lxxxiv, 699.
GERARD AND FORBES. 1928. This Journal, lxxxvi, 178.
GILDEMEISTER. 1908. Pflüger's Arch., cxxiv, 447.
GOTCH. 1900. Schäfer's Text book of physiology. London, ii, 540.
GOTCH AND BURCH. 1898. Proc. Roy. Soc., lxiii, 300.
GRAHAM. 1930. Journ. Pharm. Exper. Therap., Proc. Pharm. Soc.
HERING. 1884. Sitzungsber. d. k. Akad. der Wissen., Abt. 3, lxxxix, 137.
KATO, HAYASHI, OTA, NAKAYAMA, TAMURA, TAKEUCHI, KANAI AND MATSUYAMA. 1929. This Journal, lxxxix, 471.
LEVIN. 1927. This Journal, lxiii, 113.
VERZÁR. 1924. Pflüger's Arch., ccvi, 703.
1926. Pflüger's Arch., ccxi, 244.
WORONZOW. 1924. Pflüger's Arch., ccvi, 1.
ZOTTERMAN. 1928. Journ. Physiol., lxvi, 181.

# HEMOLYSIS OF THE ERYTHROCYTES OF AMPHIBIA

HARRY A. CHARIPPER[1]

*From the Department of Biology, New York University*

Received for publication March 13, 1930

The action of saponin, sodium taurocholate and sodium oleate on mammalian red cells has been extensively studied. Aside from the results of the action of these simple hemolysins on chicken blood (1) there is no record of quantitative investigations on nucleated red cells. This paper is concerned with the hemolysis of erythrocytes from Amphibia. Three species of animals are used; Siren lacertina, Necturus maculosus and Rana catesbeiana. They represent the three most divergent forms available.

METHODS. The methods used in this work are practically identical with those already fully described in the literature by Ponder (2), Yeager (3), and Shattuck (4). The methods described by Shattuck have been used, for the most part, as a guide for the present investigation. The ordinary strength suspension, 2.5 per cent blood cells, was found inadequate in this work as in that of Shattuck. A triple strength suspension, 7.5 per cent blood cells, gives a more distinct end-point for the lysin saponin in the case of Necturus, for the sodium taurocholate and sodium oleate hemolysis of the cells from Rana catesbeiana and for all three lysins in the case of Siren lacertina. A double strength suspension, 5 per cent blood cells, gives a satisfactory end-point for the sodium taurocholate and sodium oleate hemolysis of the cells from Necturus and also for the hemolysis by saponin of the blood cells from Rana catesbeiana.

The red cells of Rana catesbeiana are suspended in a 0.65 per cent sodium chloride solution and the cells of Siren lacertina in a 0.85 per cent saline solution; no hemolysis is observed in either of these suspensions. The erythrocytes from Necturus maculosus are suspended in a 0.2 per cent saline solution; after five or six hours a slight amount of hemolysis occurs in this suspension. In every case the suspensions are made from freshly drawn oxalated or citrated blood and used within an hour or two after preparation. All three lysins are tested upon separate portions of the same blood suspension. The frog and Siren blood are obtained from cardiac punctures while the blood of Necturus is drawn directly from the branchial circulation (4).

[1] Instructor, Department of Biology, Washington Square College.

During hemolysis or cytolysis by all three lysins the end-point is difficult to determine because of the nuclei and ghosts which remain suspended in the system and prevent the suspension from giving the clear end-point that is obtained in the case of suspensions of non-nucleated red cells. A definite end-point can be determined with practice, however, and is easily checked by examining a portion of the suspension under the microscope. Both saponin and sodium taurocholate cause hemolysis only, leaving ghosts and nuclei suspended in the system. Sodium oleate produces both cytolysis and karyolysis making it possible to obtain two end-points, the first for hemolysis and the second for karyolysis. In the ordinary concentrations of lysin used in this work the nucleus is not destroyed until hours after cytolysis. In order to get karyolysis within reasonable time limits it is necessary to use concentrations of lysin of such an order that cytolysis occurs almost instantaneously. It would be necessary to make two separate series of dilutions, therefore, in order to obtain both end-points. The present work concerns itself entirely with cytolysis.

RESULTS. In the tables which follow will be found representative curves. In every case the values for the standard (blood of man) have been included for the sake of completeness. The observed curves are fitted by means of the expression

$$t = \frac{1}{k} \log \frac{c}{c - x}$$

The values for $k$ are given as such in the tables. The values for $x$ are those given as $C\infty$. The asymptotes are given as dilution at infinity ($\delta\infty$).

*1. Hemolysis by saponin.* A. Siren lacertina

| $\delta$ | t-OBSERVED | | t-CALCULATED | | SIREN | MAN |
|---|---|---|---|---|---|---|
| | Siren | Man | Siren | Man | | |
| 10,000 | 0.4 | 0.9 | 0.6 | 0.98 | $\delta_\infty$ = 50,000 | 50,000 |
| 20,000 | 1.0 | 2.2 | 1.4 | 2.2 | $C_\infty$ = 0.04 | 0.04 |
| 30,000 | 2.1 | 4.0 | 2.1 | 4.0 | k = 0.3704 | 0.2273 |
| 40,000 | 4.4 | 6.8 | 4.3 | 7.1 | (See fig. II) | |

B. Necturus maculosus

| $\delta$ | t-OBSERVED | | t-CALCULATED | | NECTURUS | MAN |
|---|---|---|---|---|---|---|
| | Necturus | Man | Necturus | Man | | |
| 10,000 | 1.25 | 0.4 | 1.6 | 0.65 | $\delta_\infty$ = 51,000 | 41,000 |
| 20,000 | 3.9 | 1.5 | 3.7 | 1.6 | $C_\infty$ = 0.0392 | 0.049 |
| 30,000 | 6.9 | 4.0 | 6.4 | 4.0 | k = 0.1370 | 0.4239 |
| 40,000 | 10.9 | 10.3 | 11.2 | 9.2 | | |
| 50,000 | 26.5 | | 28.6 | | (See fig. I) | |

C. Rana catesbeiana

| δ | t-OBSERVED | | t-CALCULATED | | RANA | MAN |
|---|---|---|---|---|---|---|
| | Rana | Man | Rana | Man | | |
| 10,000 | 0.5 | 0.2 | 0.56 | 0.39 | $\delta_\infty$ = 68,000 | 52,500 |
| 20,000 | 1.3 | 0.75 | 1.65 | 0.85 | $C_\infty$ = 0.029 | 0.038 |
| 30,000 | 2.6 | 1.2 | 2.79 | 1.6 | k = 0.2083 | 0.6250 |
| 40,000 | 4.3 | 2.6 | 4.26 | 2.6 | | |
| 50,000 | 6.7 | 6.0 | 6.37 | 6.0 | | |
| 60,000 | 10.9 | | 10.29 | | (See fig. III) | |

The values of $k$, the velocity constant, for hemolysis by saponin of the blood of the different amphibia varies from 0.1370 to 0.3704 while the velocity constant for the standard (man) varies from 0.2273 to 0.625. It is also interesting to note that the time-dilution curves for siren and man (fig. 2) approach the same asymptote with different velocity constants while the curves for frog and man (fig. 3) as well as the curves for Necturus and man (fig. 1) approach different asymptotes with different velocity constants and cross en route.

*2. Hemolysis by sodium taurocholate.* A. Siren lacertina

| δ | t-OBSERVED | | t-CALCULATED | | SIREN | MAN |
|---|---|---|---|---|---|---|
| | Siren | Man | Siren | Man | | |
| 1,000 | 1.3 | 1.0 | 1.4 | 0.83 | $\delta_\infty$ = 6,200 | 5,000 |
| 2,000 | 3.0 | 1.9 | 3.4 | 1.88 | $C_\infty$ = 0.323 | 0.4 |
| 3,000 | 5.3 | 2.8 | 4.8 | 3.4 | k = 0.125 | 0.2703 |
| 4,000 | 8.6 | 7.0 | 8.3 | 5.95 | | |
| 5,000 | 12.1 | | 13.1 | | (See fig. VI) | |

B. Necturus maculosus

| δ | t-OBSERVED | | t-CALCULATED | | NECTURUS | MAN |
|---|---|---|---|---|---|---|
| | Necturus | Man | Necturus | Man | | |
| 300 | 1.5 | 0.4 | 1.5 | 0.31 | $\delta_\infty$ = 2,000 | 2,100 |
| 600 | 3.2 | 1.0 | 3.2 | 0.61 | $C_\infty$ = 1.00 | 0.95 |
| 900 | 5.5 | 1.6 | 5.4 | 1.2 | k = 0.1111 | 0.4762 |
| 1,200 | 8.3 | 2.0 | 8.0 | 1.7 | | |
| 1,500 | 12.3 | 3.0 | 12.4 | 2.8 | | |
| 1,800 | 28.2 | 4.0 | 20.8 | 4.1 | | |

C. Rana catesbeiana

| δ | t-OBSERVED | | t-CALCULATED | | RANA | MAN |
|---|---|---|---|---|---|---|
| | Rana | Man | Rana | Man | | |
| 1,000 | 0.85 | 0.93 | 1.9 | 0.94 | $\delta_\infty$ = 4,200 | 5,000 |
| 2,000 | 4.8 | 2.0 | 4.5 | 2.1 | $C_\infty$ = 0.48 | 0.4 |
| 3,000 | 8.7 | 3.1 | 8.7 | 3.8 | k = 0.1449 | 0.2381 |
| 4,000 | 22.0 | 6.8 | 22.2 | 6.8 | | |

The tabulated results for the hemolysis by sodium taurocholate show that the values of $k$ for the animals used vary from 0.1111 to 0.1449 while

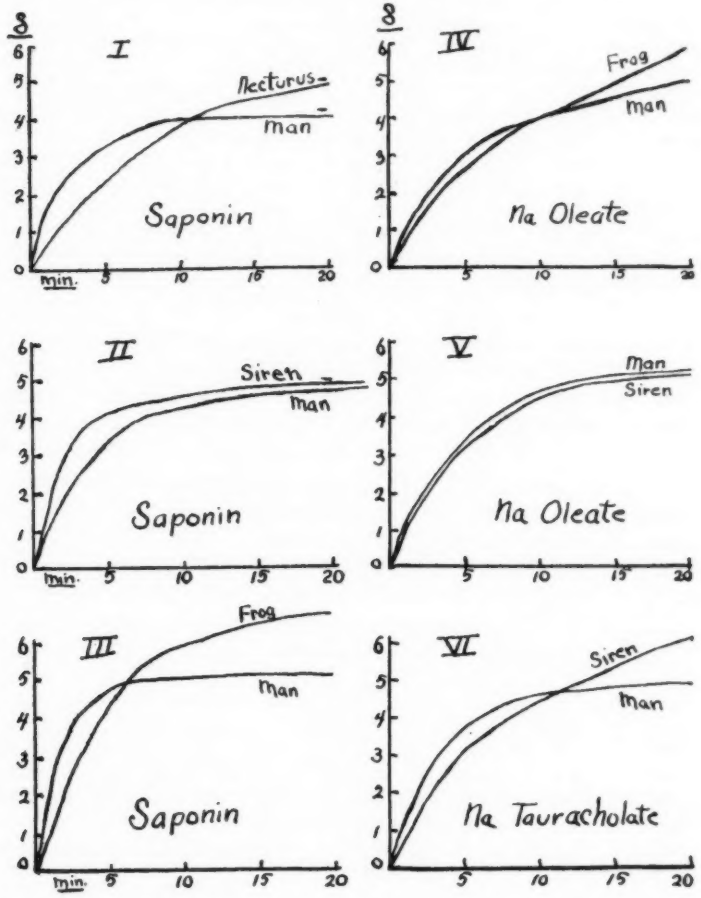



Plate 1. Time-dilution curves of nucleated red cells of amphibia. Hemolysis

Dilutions ($\delta$) for saponin in tens of thousands.
Dilutions for sodium oleate and sodium taurocholate in thousands.

the values of $k$ for the standard vary from 0.2703 to 0.4762. The time-dilution curves for Necturus and man approach approximately the same asymptote with different velocity constants. The curves for frog and

man approach different asymptotes with different values of $k$ as do those for siren and man (fig. 6). In the latter case crossing occurs.

*3. Hemolysis by sodium oleate.* A. Siren lacertina

| $\delta$ | t-OBSERVED | | t-CALCULATED | | SIREN | MAN |
|---|---|---|---|---|---|---|
| | Siren | Man | Siren | Man | | |
| 1,000 | 0.6 | 0.4 | 1.08 | 1.0 | | |
| 2,000 | 1.9 | 2.1 | 2.35 | 2.3 | $\delta_\infty$ = 5,200 | 5,300 |
| 3,000 | 4.1 | 4.0 | 4.27 | 4.0 | $C_\infty$ = 0.38 | 0.37 |
| 4,000 | 7.4 | 6.7 | 7.1 | 6.7 | k = 0.2000 | 0.2000 |
| 5,000 | 15.9 | 13.0 | 14.97 | 12.9 | (See fig. V) | |

B. Necturus maculosus

| $\delta$ | t-OBSERVED | | t-CALCULATED | | NECTURUS | MAN |
|---|---|---|---|---|---|---|
| | Necturus | Man | Necturus | Man | | |
| 500 | 0.2 | 0.5 | | | $\delta_\infty$ = 7,500 | 6,100 |
| 1,000 | 0.4 | 1.2 | 0.51 | 0.8 | $C_\infty$ = 0.26 | 0.33 |
| 2,000 | 1.1 | 1.9 | 1.1 | 1.8 | k = 0.2703 | 0.2273 |
| 3,000 | 1.5 | 2.5 | 1.9 | 2.9 | | |
| 4,000 | 2.9 | 4.6 | 2.7 | 4.7 | | |
| 5,000 | 3.9 | 7.7 | 3.9 | 7.7 | | |
| 6,000 | 5.4 | 18.5 | 5.9 | 20.7 | | |
| 7,000 | 12.1 | | 10.0 | | | |

C. Rana catesbeiana

| $\delta$ | t-OBSERVED | | t-CALCULATED | | RANA | MAN |
|---|---|---|---|---|---|---|
| | Rana | Man | Rana | Man | | |
| 500 | 0.13 | 0.433 | | | | |
| 1,000 | 1.9 | 1.4 | 1.6 | 1.3 | $\delta_\infty$ = 6,000 | 5,200 |
| 2,000 | 3.4 | 2.9 | 3.4 | 2.8 | $C_\infty$ = 0.33 | 0.38 |
| 3,000 | 6.1 | 5.2 | 5.9 | 5.1 | k = 0.1176 | 0.166 |
| 4,000 | 9.3 | 7.8 | 9.3 | 8.6 | | |
| 5,000 | 13.9 | 17.3 | 15.2 | 18.0 | (See fig. IV) | |

The results of the hemolysis of amphibian blood by sodium oleate show the velocity constant to vary from 0.1176 to 0.2703 while the values of $k$ for the standard vary from 0.16 to 0.2273. The time-dilution curves for siren and man (fig. 5) approach the same asymptote with the same velocity constant. The curves for Necturus and man and for frog and man (fig. 4) approach different asymptotes with different velocity constants. In the latter case crossing occurs.

RESISTANCE OR R VALUES. The resistance constants are obtained by the ratio $C_1\infty/C_2\infty$, $C_1\infty$ being the asymptotic value for the animal while $C_2\infty$ is the asymptotic value for the standard. Since Ponder (5) has shown

that the $R$ values are absolute values and are comparable, they afford a means of directly comparing the resistance of the blood of different animals. The $R$ values for the animals used in these experiments are tabulated below.

| | NECTURUS | SIREN | RANA |
|---|---|---|---|
| Saponin | 0.80 | 1.00 | 0.76 |
| Sodium taurocholate | 1.05 | 0.80 | 1.20 |
| Sodium oleate | 0.78 | 1.03 | 0.87 |

It should be noted that when the animals are placed in a descending series according to their $R$ values for saponin hemolysis, they fall in an ascending phylogenetic order; Siren lacertina, Necturus maculosus, Rana catesbeiana. It would be interesting to determine the resistance values for saponin hemolysis of the blood of other species of amphibia to determine whether they fall in order with reference to their phylogenetic position.

*Microscopic observations.* The erythrocytes of all three forms of Amphibia studied appear ellipsoidal in the saline suspensions used. The nucleus appears as a biconvex body in the center of the cell, sufficiently thicker than the cytoplasmic portion of the cell to cause a perceptible convexity in the cell outline when the cell is viewed on edge. Upon addition of the lysin the cell tends to round up, to assume a spheroidal shape and to fade from view leaving the nucleus in the center of a spheroidal ghost-like cell. These observations, which are true for all three lysins, are similar to the observations of Shattuck on chicken blood and seem to indicate a similarity in the hemolysis of the nucleated red cell from Amphibia, the nucleated red cell of the chicken and the non-nucleated blood cells of the mammal.

Discussion. In a recent preliminary report on the use of equations to describe the action of simple hemolysins, Ponder and Yeager (6) have found that when time-dilution curves are extended over periods as long as three hundred (300) minutes the original expression, used to fit the short time curves, requires modification.

The original expression

$$dx/dt = k\,(c-x)$$

is modified to

$$dx/dt = k\,(c\text{-}x)^n$$

and $n$ is taken to equal $1/p$. When $n$ is taken to equal 1 the original and the modified forms are identical, and

$$t = \frac{1}{k} \log \frac{c}{c - x}$$

may still be used to describe the action of the simple hemolysins over the relatively short periods of time. It would be interesting, however, to extend the observations on the blood of Amphibia over greater lengths of time and determine the amount of discrepancy, if any, between the application of the new and the old expression.

Considering the fitting of the observed time-dilution curves by the expression hitherto used, it is interesting to know that it is relatively easy to obtain good fits for curves on the blood from Amphibia, camels (7) and chicken. The erythrocytes of these three different bloods have a common factor in their elliptical shape. The red blood cells of the Amphibia and chicken have an additional common factor in the presence of a nucleus in each form. The calculated curves on the red cells of the Amphibia and chicken seem to be closer fits than those on the camel cells. It appears, therefore, that the contour and the morphological nature of the erythrocyte may be factors, as worthy of consideration as the nature of the cell membrane alone, in an explanation of the mechanism of hemolysis.

## CONCLUSIONS

1. Time-dilution curves are given for the hemolytic action of saponin, sodium taurocholate and sodium oleate on the erythrocytes of three different species of Amphibia: Siren lacertina, Necturus maculosus and Rana catesbeiana.

2. Resistance values for the red cells of each of the animals are given for the three lysins.

3. All three lysins produce hemolysis in the case of each animal. Sodium oleate also causes karyolysis.

4. Microscopic observation of the nucleated red cell in the presence of the lysins, for all three forms studied, show changes similar to those reported for the nucleated red cell of chicken blood and the non-nucleated red blood cell of mammals.

## BIBLIOGRAPHY

(1) Shattuck, G. E. Journ. Gen. Physiol., 1928, xii, 17.
(2) Ponder, E. Proc. Roy. Soc., Series B, 1921, xcv, 42.
(3) Yeager, J. F. Journ. Gen. Physiol., 1928, xi, 779.
(4) Charipper, H. A. and A. B. Dawson. Anat. Rec., 1928, xxxix, 301.
(5) Ponder, E. Proc. Roy. Soc., Series B, 1926, xcix, 461.
(6) Ponder, E. and J. F. Yeager. Proc. Soc. Exper. Biol. Med., 1929, xxvii, 53.
(7) Ponder, E., J. F. Yeager and H. A. Charipper. Quart. Journ. Exper. Physiol., 1928, xix, 115.

# ON THE PRESENCE OF CHOLECYSTOKININ IN THE HUMAN AND RABBIT INTESTINE

G. E. DREWYER[1] AND A. C. IVY

*From the Department of Physiology and Pharmacology, Northwestern University Medical School*

Received for publication March 13, 1930

Ivy, Kloster, Lueth and Drewyer (1) found cholecystokinin to be present in the upper intestine of the hog, dog, sheep, and cattle. They also found that a small amount could occasionally be extracted from the pyloric mucosa of the stomach of the dog and hog. They were unable to obtain the active principle from extracts of the kidney, spleen and liver.

In this paper we report the existence of cholecystokinin in the upper intestine of the human being and rabbit.

METHODS. Through the courtesy of Dr. J. P. Simonds of the Department of Pathology, we were able to obtain several human intestines from post mortems held within one or two hours after death.

The human and rabbit intestines were treated by the method of Ivy, Kloster, Lueth and Drewyer (1) to yield an "A pH 1802 powder." This product was assayed by the method of Ivy and Oldberg (2).

RESULTS. *Human intestine.* From the first two yards of the human intestine we obtained from 15 to 25 "dog" doses of cholecystokinin which compares favorably to the yield of the hog intestine (fig. 1a). Two human intestines were extracted.

*Rabbit intestine.* The entire small intestine of six rabbits was extracted. They yielded an average of nine "dog" doses of cholecystokinin and twenty-five doses of secretin (fig. 1b).

## CONCLUSION

Cholecystokinin can be extracted from rabbit and human intestinal mucosa.

## BIBLIOGRAPHY

(1) IVY, KLOSTER, LUETH AND DREWYER. 1929. This Journal, xci, 336.
(2) IVY AND OLDBERG. 1928. This Journal, lxxxvi, 599.

[1] Eli Lilly Fellow.

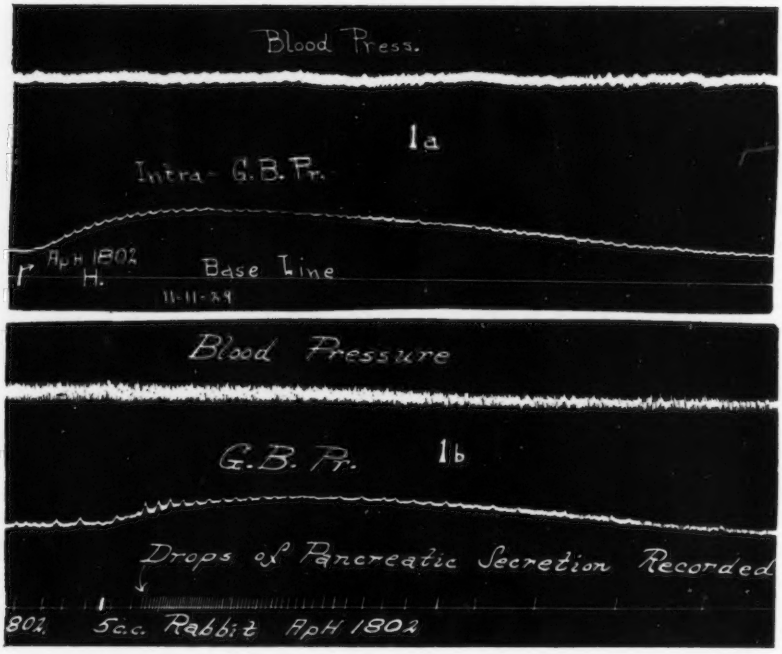



Fig. 1. Tracing "1a" shows the response of the dog's gall bladder to cholecystokinin, "A pH 1802", extracted from the human upper intestinal mucosa.

Tracing "1b" shows the response of the dog's gall bladder to cholecystokinin and secretin extracted from the intestinal mucosa of the rabbit.

# PHYSIOLOGICAL VARIATIONS IN THE CARDIAC OUTPUT OF MAN

## IX. The Effect of Breathing Carbon Dioxide, and of Voluntary Forced Ventilation on the Cardiac Output of Man[1]

ARTHUR GROLLMAN

*From the Physiological Laboratory, School of Medicine, The Johns Hopkins University*

Received for publicaton March 15, 1930

The rôle of carbon dioxide in regulating the pulmonary ventilation has led investigators to seek a relationship, if any, between the carbon dioxide content of the inspired air and the circulatory system. A voluminous literature has accumulated dealing with this subject. Our knowledge concerning the action of carbon dioxide on the circulation of normal man, however, particularly on the cardiac output, is still incomplete. Previous investigators have failed to consider the duality of the factors which might affect the circulation as a result of an increased alveolar concentration of carbon dioxide. Besides the chemical action of carbon dioxide on the blood vessels, heart, or nervous system, one has to consider the incidental mechanical effects of the increased respiratory activity which accompanies the inspiration of carbon dioxide. Indeed, as will be seen later, this mechanical action produces changes in the cardiac output as great as those which follow the inspiration of carbon dioxide. The nature of the effects of forced breathing on the cardiac output of man has not been adequately investigated, previous studies being usually limited to considerations of changes in the pulse rate and blood pressure.

It was the purpose of the present investigation to study the effect of carbon dioxide on the cardiac output and to attempt to elucidate the mode of action of this gas on the circulation. For a better understanding of this effect it was necessary also to investigate the effect of voluntary forced respiration with and without change in the alveolar carbon dioxide tension.

Methods. The experiments recorded in the present investigation were performed on young, normal individuals in the basal condition as defined in a previous paper (Grollman, 1929d). The cardiac outputs were determined by the use of acetylene[2] (Grollman, 1929a), pulse rates by palpation

[1] A preliminary report of the results presented in this paper was given at the annual meeting of The American Physiological Society, Chicago, 1930.

[2] Kroetz (Klin. Wochschr., 1930, ix, 966) has recently reported the results of his studies with acetylene (Grollman, 1929 a). He confirms the high degree of accuracy

for 15 or, where feasible, for 30 seconds, and blood pressures by the Tycos recording sphygmomanometer.

The gas mixtures used were prepared by compressing carbon dioxide and air in a tank. The resulting mixtures were then led into a *Douglas* bag from which they were inspired. This method permits testing the effect of a given mixture for any length of time. There is a latent period for which any mixture must be breathed before the maximum respiratory response is elicited (Paget, 1924), and this latent period probably also precedes the development of the maximum cardiac output with any given mixture. It is for this reason that rebreathing experiments are less suitable for eliciting the true response of the heart to a given concentration of carbon dioxide.

In the experiments on the effect of voluntary forced ventilation on the cardiac output, the subject attempted to maintain his respiratory ventilation at an increased, but uniform, rate and depth. In order to study the effect of the alveolar carbon dioxide on the cardiac output, voluntary forced breathing was also carried out by inspiring a mixture of air plus carbon dioxide. By proper adjustment of the composition of the inspired gas and of the rate and depth of ventilation, one could exert an increased respiratory effort without affecting the carbon dioxide content of the arterial blood as determined by the composition of the alveolar air. In this way, the purely mechanical effect of forced breathing could be studied without the complication of chemical changes due to increase or diminution of carbon dioxide.

Due to the stupefying effect which voluntary forced ventilation of air has on the organism, it was impossible to carry out such experiments for

---

and reproducibility which are obtainable by the use of this gas, as demonstrated in the papers of this series (Grollman, 1928, 1929, 1930). Several statements in the paper of Kroetz, however, require comment. The long neck of the bag containing the experimental gas mixtures (as pictured in Figure 1 of Kroetz's paper) is undesirable due to the interference with mixing which this uncalled-for "dead space" introduces. The addition of oxygen to the experimental gas mixture (although suggested in the original paper presenting the use of acetylene) is usually unnecessary, as the oxygen content of the samples taken for analysis are such as to give tensions corresponding to the flat portion of the oxygen-dissociation curve of blood. Kroetz states that the time necessary for doing an analysis of $CO_2$, $C_2H_2$, $O_2$ and $N_2$ is 20 to 22 minutes and that a complete determination requires 1¼ hours. I have found the gas analysis to require only about 10 minutes and thus 12 or more determinations of the cardiac output may be completed by a worker in a single day. Kroetz found the cardiac output to be about 20 per cent higher in the recumbent than in the sitting posture. In a previous paper dealing with posture (Grollman, 1928), such differences were found to be due to errors in attaining perfect homogeneous mixture in the recumbent position. These results regarding the independence of the cardiac output to posture were obtained with nitrous oxide as the foreign gas, and have been recently confirmed by the author (Grollman, This Journal, 1930, xciii, 116) both by the acetylene method and by the triple extrapolation method based on the Fick principle.

any great length of time. Extremely painful reactions such as occur after prolonged forced breathing were avoided. Nevertheless, it is quite possible that the disturbing dyspnea and feeling of suffocation which accompany the inspiration of high concentrations of carbon dioxide and the stupefying effect of forced breathing may have psychically produced part of the increase in the cardiac output which has been observed during the present work. The nature of such psychic effects on the cardiac output has been previously demonstrated (Grollman, 1929c).

The oxygen consumption during the experimental periods was determined in the usual manner by collection of the expired gas in a *Douglas* bag. Due to the small differences in composition of the inspired and expired gas, and to the vitiating effect of any non-uniformity in breathing, the determination of the oxygen consumption was in many cases fraught with error. Consequently, in those experiments in which the breathing periods were continued for only a short time, the oxygen consumption was determined in a separate experiment. The result was then used in the subsequent determination of the arterio-venous oxygen difference to give the cardiac output. The oxygen consumptions during hyperventilation recorded in tables 1, 2, and 3 of this paper show an increase over the normal due, no doubt, to the extra muscular effort involved in the respiratory movements, as was also found by Bornstein and v. Gartzen (1905) and Reach and Röder (1909).

RESULTS. Four subjects were used in all of the experiments discussed in the present paper. Since the results on all subjects were in accord with the general conclusions deduced, only typical experiments are cited in tables 1, 2, and 3 which give the results of the present investigation.

*Effect of carbon dioxide on the circulation.* In table 1 are given typical results obtained after breathing *carbon dioxide-air* mixtures for varying periods of time. Columns 7 and 8 show the rise in pulse rate and blood pressure which are similar to those noted by previous workers (Schneider and Truesdell, 1922; Goldstein and Du Bois, 1927). As shown in the last column of table 1, the cardiac output is not affected by moderate concentrations of carbon dioxide, despite the appreciable increase in the pulmonary ventilation (column 5) and the alveolar carbon dioxide tension (column 3). At higher concentrations when the pulmonary ventilation is very greatly increased the cardiac output is also increased. The conclusion to be drawn from table 1 is that *the inspiration of carbon dioxide is without effect on the circulatory rate of man until one reaches a concentration of over 6 per cent in the inspired air.*

Lindhard (1915) in three experiments on one individual found no change in the cardiac output on breathing a 4 per cent *carbon dioxide-air* mixture. Liljestrand (1919) gives results on 2 subjects, and concludes that any effect of carbon dioxide, if present, is small. Douglas and Haldane (1922)

TABLE 1

*The effect of the inspiration of carbon dioxide-air mixtures on the circulation*

| EXPERIMENT NUMBER | $CO_2$ CONTENT OF THE INSPIRED GAS | ALVEOLAR $CO_2$ CONTENT OF THE DRY GAS | TIME DURING WHICH BREATHING WAS CONTINUED | RESPIRATORY MINUTE VOLUME | OXYGEN CONSUMPTION | PULSE RATE | BLOOD PRESSURE | ARTERIOVENOUS OXYGEN DIFFERENCE | CARDIAC OUTPUT |
|---|---|---|---|---|---|---|---|---|---|
| | *volumes per cent* | *volumes per cent* | *minutes* | *liters* | *cc. per minute* | *per minute* | *mm. Hg* | *cc. per liter* | *liters per minute* |
| 1 | 0.04 | 4.59 | | 8.6 | 240 | 59 | 100/67 | 55 | 4.4 |
| | 1.52 | 5.27 | 15 | 10.1 | 245 | 60 | 102/69 | 57 | 4.3 |
| 2 | 0.04 | | | 6.3 | 200 | 64 | | 55 | 3.6 |
| | 1.82 | 6.03 | 5 | 7.8 | 200 | 64 | | 57 | 3.7 |
| | 1.82 | | 20 | 7.8 | 200 | 65 | | 54 | 3.7 |
| | 1.82 | | 37 | 7.9 | 200 | 67 | | 57 | 3.5 |
| 3 | 0.04 | 4.96 | | 8.7 | 240 | 60 | 93/55 | 60 | 4.0 |
| | 3.52 | 5.51 | 11 | 13.4 | 245 | 60 | | 64 | 3.8 |
| | 3.52 | 5.61 | 27 | 14.2 | 246 | 60 | | 60 | 4.1 |
| | 3.52 | 5.60 | 40 | 14.4 | 250 | 64 | 88/55 | 65 | 3.8 |
| 4 | 0.04 | | | 8.9 | 250 | 74 | 101/67 | 60 | 4.1 |
| | 5.93 | | 15 | 24.0 | 268 | 80 | 122/78 | 56 | 4.8 |
| 5 | 0.04 | | | 8.7 | 240 | 60 | 108/77 | 55 | 4.4 |
| | 6.05 | 6.98 | 8 | 18.4 | 252 | 60 | 122/78 | 55 | 4.6 |
| | 6.05 | | 3 | 18.8 | 252 | 60 | 119/82 | 60 | 4.2 |
| | 0.04 | | 15 | 8.6 | 240 | 60 | 112/85 | 58 | 4.1 |
| 6 | 0.04 | 4.59 | | 8.5 | 268 | 59 | | 53 | 5.0 |
| | 6.90 | 7.26 | 15 | 37.5 | 326 | 70 | | 53 | 6.2 |
| 7 | 0.04 | | | 8.5 | 240 | 63 | | 60 | 4.0 |
| | 7.49 | 8.06 | 4 | 31.2 | 285 | 70 | 112/80 | 57 | 5.0 |
| | 7.49 | 8.04 | 15 | 33.2 | 285 | 78 | 126/— | 53 | 5.4 |
| | 0.04 | | 10 | | 240 | 70 | 118/84 | 57 | 4.2 |
| 8 | 0.04 | 5.42 | | 6.0 | 242 | 66 | 102/66 | 60 | 4.0 |
| | 7.61 | 8.28 | 10 | 41.0 | 266 | 99 | 110/75 | 50 | 5.3 |
| | 7.61 | 8.35 | 25 | 41.0 | 266 | 99 | 120/72 | 47 | 5.7 |
| 9 | 0.04 | 5.42 | | 6.3 | 200 | 66 | 98/63 | 50 | 4.0 |
| | 9.25 | 9.37 | 3 | 30.2 | 240 | 99 | 117/82 | 43 | 5.6 |
| | 9.25 | 9.40 | 5 | 31.5 | 240 | 99 | 111/— | 42 | 5.7 |

found a 5 per cent *carbon dioxide-air* mixture to have no effect on the one subject whom they studied. They explain their result by assuming that the increase in cardiac output which they expected was counterbalanced by the decrease occasioned by the higher oxygen tension of the arterial blood

which results from the increased respiration. This latter assumption concerning the effect of slight changes in the oxygen content of the arterial blood on the cardiac output is based on the experiments of Dautrebrande and Haldane (1921) which, however, have not been substantiated by the author (Grollman, 1930).

St. Janoušek (1928) found that concentrations of carbon dioxide up to 4 per cent decreased the cardiac output, as measured by the ethyl iodide method while higher concentrations raised it. Schneider and Truesdell (1922), using Henderson's (1905) recoil-board method, could detect no

TABLE 2

*The effect of voluntary forced respiration on the circulation*

| EXPERIMENT NUMBER | TIME DURING WHICH BREATHING WAS CONTINUED | RESPIRATORY MINUTE VOLUME | OXYGEN CONSUMPTION | PULSE RATE | BLOOD PRESSURE | ARTERIO-VENOUS OXYGEN DIFFERENCE | CARDIAC OUTPUT |
|---|---|---|---|---|---|---|---|
| | *minutes* | *liters* | *cc. per minute* | *per minute* | *mm. Hg* | *cc. per liter* | *liters per minute* |
| 1 | Control | 8.4 | 240 | 76 | 105/68 | 63 | 3.8 |
| | 10 | 30.5 | 280 | 85 | 100/72 | 60 | 4.7 |
| | 6 | 40.0 | 300 | 91 | 88/63 | 58 | 5.2 |
| | 1½ | 60.0 | 340 | | | 70 | 4.9 |
| 2 | Control | 8.5 | 240 | 76 | 113/68 | 63 | 3.8 |
| | 5 | 38.8 | 290 | 114 | 123/68 | 58 | 5.0 |
| | 1½ | 60.0 | 240 | | | 62 | 5.5 |
| 3 | Control | 6.3 | 200 | 65 | 90/58 | 59 | 3.4 |
| | 7 | 24.3 | 230 | 76 | 96/65 | 54 | 4.3 |
| 4 | Control | 8.4 | 260 | 68 | 100/65 | 60 | 4.3 |
| | 5 | 30.9 | 300 | 80 | 113/62 | 65 | 4.6 |
| | 4 | 32.5 | 305 | 81 | 113/68 | 68 | 4.5 |
| 5 | Control | 8.4 | 244 | 64 | 107/66 | 61 | 4.0 |
| | 3 | 44.2 | 313 | 90 | 121/70 | 65 | 4.8 |

change in the cardiac output even on inspiring high concentrations of carbon dioxide. The fundamental inadequacy of these methods renders invalid the conclusions of these authors.

*The effect of voluntary forced breathing and the consequent reduction in the carbon dioxide content of the arterial blood on the circulation.* The increased cardiac outputs observed after inspiring relatively high concentrations of carbon dioxide might be due to a number of causes. Further experiments were, therefore, carried out to determine the extent to which the circulatory changes were produced by the action of carbon dioxide and to what

extent they resulted from the mechanical effects of the increased ventilation.

In table 2 are given the results of a series of determinations in which the rate and depth of respiration of atmospheric air was voluntarily increased with a consequent reduction of the alveolar carbon dioxide tension. The results indicate that this procedure increases the pulse rate, increases the

TABLE 3

*The effect of voluntary forced respiration of a carbon dioxide-air mixture, on the circulation*

| EXPERIMENT NUMBER | TIME DURING WHICH FORCED BREATHING WAS CONTINUED | ALVEOLAR $CO_2$ CONCENTRATION (DRY GAS) | RESPIRATORY MINUTE VOLUME | OXYGEN CONSUMPTION | PULSE RATE | BLOOD PRESSURE | ARTERIO-VENOUS OXYGEN DIFFERENCE | CARDIAC OUTPUT |
|---|---|---|---|---|---|---|---|---|
| | *minutes* | *per cent* | *liters* | *cc. per minute* | *per minute* | *mm. Hg* | *cc. per liter* | *liters per minute* |
| 1 | Control | 4.59 | 8.4 | 244 | 64 | 107/66 | 61 | 4.0 |
| | 2.2 | 4.35 | 42.6 | 309 | 84 | 118/— | 70 | 4.4 |
| | 2.6 | 4.43 | 40.9 | 306 | 76 | 122/76 | 61 | 5.0 |
| | 4.5 | 4.45 | 46.5 | 317 | 92 | 116/73 | 61 | 5.2 |
| | 5.8 | 4.67 | 39.5 | 303 | 76 | 115/68 | 69 | 4.4 |
| 2 | Control | 4.43 | 8.5 | 236 | 60 | 108/70 | 61 | 3.9 |
| | 10 | 4.51 | 46.6 | 306 | 66 | 116/74 | 67 | 4.6 |
| | 5 | 4.88 | 34.3 | 286 | 72 | 112/72 | 58 | 4.9 |
| | 4 | 4.69 | 31.6 | 276 | 72 | 111/78 | 62 | 4.5 |
| | Control | 4.45 | 8.5 | 234 | 60 | 108/72 | 59 | 3.9 |
| 3 | Control | 4.51 | 8.4 | 240 | 76 | 108/70 | 55 | 4.4 |
| | 6 | 2.62 | 47.4 | 315 | 87 | 117/— | 65 | 4.8 |
| | 5 | 2.75 | 54.0 | 325 | 87 | 122/70 | 59 | 5.9 |
| | 2.5 | 2.89 | 48.8 | 318 | 87 | 131/64 | 74 | 4.3 |
| 4 | Control | 6.07 | 6.3 | 200 | 65 | 90/58 | 59 | 3.4 |
| | 10 | 6.22 | 14.2 | 210 | 72 | 91/60 | 57 | 3.7 |
| 5 | Control | 4.85 | 8.4 | 260 | 68 | 108/63 | 60 | 4.3 |
| | 4 | 7.64 | 48.0 | 335 | 84 | 121/82 | 62 | 5.4 |
| | 4.3 | 6.58 | 42.1 | 325 | 84 | 123/80 | 59 | 5.5 |

blood pressure (except in experiment 1 where a reduction occurs), and increases the cardiac output to a moderate degree. If one compares these results with those of table 1, one finds that they are strikingly similar, except, perhaps, for less uniformity in the results of table 2. This lack of uniformity with forced breathing is attributed to the inability to maintain a perfectly uniform rate of respiration by voluntary forced breathing as compared to the involuntary but uniform results which follow the inspiration of *carbon dioxide-air* mixtures.

In so far as one is able to conclude from the results of table 2, the effect of forced ventilation with reduction of the carbon dioxide tension of the alveolar air on the cardiac output is about the same as the effect of the increased ventilation occurring with the inspiration of carbon dioxide-air mixtures. In the former experiments, however, as in the latter, one has to differentiate two factors—a chemical and a mechanical. The reduction of the alveolar carbon dioxide tension might act as a chemical factor in altering the circulation while the increased respiratory effort might exert a mechanical effect.

*The effect of forced breathing without change in the alveolar carbon dioxide on the circulation.* In order to determine which of the above mentioned factors produces the circulatory changes recorded in tables 1 and 2, a third series of experiments was conducted in which a carbon dioxide-air mixture was voluntarily inspired at a rate which resulted in only moderate changes in the concentration of the alveolar air. These results are recorded in table 3. It will be observed that the changes in pulse, blood pressure, and cardiac output are, in general, similar to those recorded in tables 1 and 2. Since the only factor involved in the experiments of table 3 is the mechanical effect of the increased respiratory effort, it might be concluded that this factor is also predominant in producing the changes observed in tables 1 and 2. The chemical factor, whether produced either by an increase or by a reduction in the carbon dioxide of the alveolar air (and consequently of the arterial blood and of the tissues) would, in accord with this view, play little part in producing the observed changes in the circulation. It might, on the other hand, also be argued that it is only fortuitous that the observed changes, which are the summation of a number of factors, should give approximately the same results (in tables 1, 2, and 3). The most pertinent fact in favor of the latter viewpoint is the difference in the effect of carbon dioxide and of forced ventilation of air on other factors of the circulation, such as on the blood pressure, peripheral circulation, *etc.* In so far as the present investigation is concerned, however, there is a remarkable similarity in the results of all the factors studied, independent of the carbon dioxide tension of the alveolar air.

It will be noted that the arterio-venous oxygen differences during hyperventilation, as given in tables 1, 2, and 3, do not, in general, deviate far from the control values. The generally accepted notion at present is that the cardiac output is a function of the metabolism, and increases during mild exercise with the oxygen consumption. The observed increases in cardiac output of tables 1, 2, and 3 would, in accordance with this view, be attributable to the increased muscular effort of hyperventilation. Preliminary experiments by the author have not, however, substantiated the commonly accepted view. The observed increases in cardiac output must be considered as a true increase in activity of the heart in re-

sponse to hyperventilation. From the point of view of the muscular exercise involved, the increase in oxygen consumption accompanying the increased respiratory movements should have resulted in an increase in the arterio-venous oxygen difference, without change in the cardiac output, as was observed in the case of postural changes (Grollman, 1928).

Discussion. That the respiratory movements play an essential part in returning the venous blood to the right heart has been generally accepted since this conception was first advanced by Barry, (1825) and Poiseuille (1830). If one increases the intensity and frequency of these respiratory movements (without altering the carbon dioxide tension of the blood as in the experiments of table 3), one would expect at first the return of a volume of blood, much greater than that which occurs with normal respiration. If one accepts the view of an inadequately filled heart and of the regulation of the cardiac output by the venous return there should follow, as a result of increased respiratory efforts, an immediate increase in the cardiac output. This increase, however, is transient for the rapid depletion of the venous reservoir in the thorax and abdomen brings to an end the momentarily increased return of blood to the heart. In order to maintain a continued increase in the cardiac output, the blood flow through the periphery would have to be correspondingly increased. This could occur either by vasodilatation, by increased arterial bood pressure, by diminished venous pressure, or by a combination of any of these factors. The transient nature of the great increase in cardiac output which occurs during the first few forced respirations explains the great increase in the blood flow through the lungs which has been described by Lindhard (1915). This great increase is not continued because of the depletion of the venous store and the failure of adjustments to occur in other parts of the circulatory system which would permit the continuance of this greatly augmented cardiac output.

In the case of the increased respiratory effort accompanied by an increase in the carbon dioxide tension of the blood (as in table 1) or by a decrease (as in table 2), one must also consider the possible effect of these chemical changes on the vascular system. Stewart (1911) and Schneider (1930) have demonstrated a decrease in the blood flow through the arm during voluntary forced breathing, due presumably to peripheral vasoconstriction resulting from loss of carbon dioxide. Itami (1912), Fleisch (1918), Dale and Evans (1922), and Beckmann (1929) have described similar effects of carbon dioxide on the blood vessels. The results of the present investigation would indicate that any change in the circulation due to vasomotor changes is small compared to the general increase due to the mechanical causes described above. Moreover, the observations cited are limited to the blood flow through skin, resting muscle, and bone, and show nothing regarding the flow through the brain and viscera whose condition will determine the total cardiac output which we are considering at present.

When the alveolar carbon dioxide tension is maintained constant, as in the experiments of table 3, vascular or cardiac effects due to chemical action would be unexpected. The increased cardiac outputs observed after some minutes of continued forced breathing would result from the combination of a continued increased pulse rate and arterial pressure (as observed in table 3) and the continued diminution in the venous pressure (Eyster, 1929). There is thus a sustained increase in the mechanical force which propels the blood through the capillaries and it is this sustained force to which we must attribute the increased cardiac outputs observed on forced ventilation with a constant carbon dioxide content of the alveolar air. This same force probably also produces the observed changes in the cardiac output when the carbon dioxide content of the arterial blood is not maintained constant.

The fact that pulse rate changes occur on hyperventilation without change in the carbon dioxide content of the alveolar air (as shown in table 3) indicates the existence of factors, other than chemical changes in the blood, which can affect this function in normal man. This change in pulse rate is probably brought about as a reflex (similar to that described by Bainbridge, 1915) initiated by the pressure changes, in the *venae cavae* and *atria*, occasioned by the mechanical action of the respiratory movements during hyperventilation.

The effects of forced ventilation on the pulse and blood pressure of man have been recently discussed by Collier, Densham, and Wells (1927), Vincent and Thompson (1928), Raab (1929) and Schneider (1930). There is an extreme diversity in the results of these authors which may be due to several causes. Besides the possible unreliability of the experimental methods employed, one must consider the extreme variability in the respiratory mechanism of different individuals. This is particularly true of voluntary forced breathing and has been considered by Lindhard (1915). If one accepts the view, supported by the present work, of the predominance of the mechanical factor in producing the circulatory changes accompanying hyperventilation, it is quite plausible to expect the wide variations in pulse rate and blood pressure changes which have been observed by different workers.

The effect of carbon dioxide on the cardiac output has been studied for the most part in animals other than man. Marshall (1926) found that concentrations of carbon dioxide above about 5 per cent diminished the cardiac output of normal unanesthetized dogs while lower concentrations were without effect. The pulse rate, contrary to the result observed in man, decreased as was also noted by Itami (1912) and Eppinger, Papp, and Schwarz (1927) who worked with anesthetized dogs. The latter authors, however, both found marked increases in the cardiac output of their animals. Riml (1929), likewise, found that carbon dioxide increased the

cardiac output of anesthetized rabbits. The opposite effect of carbon dioxide on the pulse rate of man and dog indicates a species difference in the reaction of this function. The differences observed by Marshall and by Itami, Eppinger, Papp, and Schwarz, and Riml may be explained as being due to the use of an anesthetic by the latter authors. The difference between the results on unanesthetized man and dog may readily be due to the difference in the mechanical effect which increased ventilation exerts on the circulatory system of the two animals. If the observed effects of carbon dioxide are not due to the chemical action of this gas but rather to the mechanical effect which the hyperventilation exerts on the circulatory system, one can readily understand why dog and man should react so differently.

The same diversity in results as regards man and the lower animals is also encountered when one considers the previous studies of the effect of forced ventilation on the circulation. Thus Bainbridge (1920), in anesthetized animals with the chest exposed, found that inhalation of carbon-dioxide-enriched air prevented the rise in pulse rate which accompanies the increased respiratory ventilation in such animals. The results of table 3, of this paper, obviously show a different reaction in normal man as regards this effect on the pulse rate. Since anesthesia may markedly alter the reaction of the circulatory system to conditions to which it is exposed. further discussion of the conclusions adduced by previous workers, who have limited their experiments to anesthetized animals, is unnecessary. The available experimental evidence indicates that the nervous system of anesthetized animals reacts to changes in carbon dioxide content and pH which are without effect in unanesthetized animals.

In passing it may be noted that the results of the present investigation give no evidence for the theory of the hydrogen ion control of the circulation as advanced by Boothby (1915). Boothby's (1912) conclusion that the absence of apnea after forced breathing is due to a compensating diminution of the circulation is also disproven by the present work. One of the subjects cited in tables 2 and 3 consistently failed to show an apnea after forced breathing, but the cardiac output was nevertheless found to be elevated. Moreover, unlike Boothby's subject, the blood pressure of this subject consistently rose during violent respiration.

The present work also shows that moderate hyperventilation does not cause the great circulatory failure demanded by Henderson's (1910) theory on the acapnic origin of shock. The marked subjective symptoms observed on violent forced breathing are to be attributed to changes in the periphery (due to loss of carbon dioxide with a consequent shift in the acid-base equilibria) and not to a circulatory failure as has been maintained. It is quite possible, of course, that long continued hyperventilation (as in the experiments of Henderson and Haggard, 1918) with consequently

marked changes in the blood and tissues, might well cause a slowing of the circulation, but such profound changes are not encountered in the experiments usually carried out on man, nor probably even under abnormal conditions as in excessive pain.

The results of the present study illustrate how easily one may be deluded in estimating the change in cardiac output from other circulatory changes. Indeed, there does not seem to be any close correlation between the cardiac output and any one single factor of the circulatory system and it is necessary to know many factors before one can visualize the complete changes which are occurring in the organism. Concerning the disproportionate and often contrary changes between the pulse rate and the cardiac output comment has already been made (Grollman, 1929e, 1930).

The venous pressure cannot be taken as a criterion of the cardiac output. Thus, after the ingestion of food where marked increases in cardiac output occur (Grollman, 1929b), the venous pressure remains unchanged (Eyster, 1929). This independence is easily understood when we consider the vasodilatation of the splanchnic areas which follow the ingestion of food. During hyperventilation Henderson, Prince, and Haggard (1918) obtained marked reductions (80 to 100 mm. of blood) of the venous pressure which were maintained for 2.5 to 6 minutes after cessation of the breathing. Kroetz (1922) by a more accurate method of measurement obtained a much smaller drop (7–25 mm.). Carbon dioxide, on the other hand, according to Schneider and Truesdell (1922), always causes a rise in the venous pressure. The cardiac output, however, as we have seen, increases in both of these cases. There is thus no generally applicable, simple relationship between venous pressure and cardiac output.

As regards peripheral changes in the arm, Schneider and Truesdell (1922) observed a fall in the blood flow through the hand while breathing carbon dioxide. Stewart (1911) and Schneider (1930) observed a fall after forced ventilation of air. Such observations on the periphery tell us nothing of concomitant changes occurring in the visceral organs nor lead us to judge of the change occurring in the cardiac output. The product of pulse pressure and pulse rate which has also been used as a measure of cardiac output is valid only where no peripheral changes occur.

The cardiac output is determined by a combination of a number of circulatory factors, viz.; pulse pressure, conditions of the peripheral vessels, venous pressure, etc.; and one cannot with certainty arrive at a conclusion concerning the cardiac output from changes in one or even several of these functions, as has been so often attempted in the past.

## SUMMARY

Carbon dioxide did not affect the circulatory system of normal man until it reached a concentration of about 6 per cent in the inspired air. Higher

concentrations caused an increase in the pulse rate, blood pressure, and cardiac output. Forced voluntary ventilation of atmospheric air also caused a moderate increase in the circulatory rate. When carbon dioxide was added to the inspired air, to avoid any change in the alveolar tension of this gas, practically similar increases were observed. Since, in the last named experiments, only the mechanical effect of hyperventilation was present, the changes observed after natural breathing of carbon dioxide-air mixtures or during forced respiration of air are also attributed to the mechanical aid which violent respiratory efforts exert on the circulation.

## BIBLIOGRAPHY


BAINBRIDGE, F. A. 1915. Journ. Physiol., 1, 65.
1920. Journ. Physiol. liv, 192.

BARRY, D. 1825. Recherches expérimentales sur les causes du mouvement du sang dans les Veines. Paris.

BECKMANN, R. 1929. Pflüger's Arch., ccxxiii, 561.

BOOTHBY, W. M. 1912. Journ. Physiol., xlv, 328.
1915. This Journal, xxxvii, 383.

BORNSTEIN, A. AND B. v. GARTZEN. 1905. Pflüger's Arch., cix, 628.

COLLIER, R. J., H. B. A. R. DENSHAM AND H. M. WELLS. 1927. Quart. Journ. Exper. Physiol., xviii, 291.

DALE, H. H. AND C. L. EVANS. 1922. Journ. Physiol., lvi, 125.

DAUTREBRANDE, L. AND J. S. HALDANE. 1921. Journ. Physiol., lv, 296.

DOUGLAS, C. G. AND J. S. HALDANE. 1922. Journ. Physiol., lvi, 69.

EPPINGER, H., F. KISCH AND H. SCHWARZ. 1927. Das Versagen des Kreislaufes. Julius Springer. Berlin, p. 175.

EYSTER, J. A. E. 1929. Clinical aspects of venous pressure. The MacMillan Co. New York.

FLEISCH, A. 1918. Pflüger's Arch., clxxi, 86.

GOLDSTEIN, J. D. AND E. L. DUBOIS. 1927. This Journal, lxxxi, 650.

GROLLMAN, A. 1928. This Journal, lxxxvi, 285.
1929a. This Journal, lxxxviii, 432.
1929b. This Journal, lxxxix, 366.
1929c. This Journal, lxxxix, 584.
1929d. This Journal, xc, 210.
1929e. This Journal, xc, 371.
1930. This Journal, xciii, 19.

HENDERSON, Y. 1905. This Journal, xiv, 287.
1910. This Journal, xxv, 310, 385.

HENDERSON, Y. AND H. W. HAGGARD. 1918. Journ. Biol. Chem., xxxiii, 355.

HENDERSON, Y., A. P. PRINCE AND H. W. HAGGARD. 1918. Journ. Pharm. Exper. Therap. xi, 203.

ITAMI, S. 1912. Journ. Physiol., xlv, 338.

KROETZ, C. 1922. Deutsch. Arch. f. klin. Med., cxxxix, 325.

LILJESTRAND, G. 1919. Skand. Arch. f. Physiol., xxxvii, 180.

LINDHARD, J. 1915. Pflüger's Arch., clxi, 233.

MARSHALL, E. K. 1926. Journ. Pharm. Exper. Therap., xxix, 167.

PAGET, P. 1928. This Journal, lxxxiii, 384.

POISEUILLE, J. L. M. 1830. Journ. de Physiol. Exper. et Path., x, 277.

RAAB, W. 1929. Zeitschr. Exper. Med., lxviii, 337.
REACH, F. AND F. RÖDER. 1909. Biochem. Zeitschr., xxii, 471.
RIML, O. 1929. Arch. f. Exper. Path. u. Pharm., cxxxix, 240.
ST. JANOUŠEK. 1928. Čas lék. čes. lxvii, 1469, quoted in Zeitschr. für Kreislaufforschung, 1929, xxi, 702.
SCHNEIDER, E. C. 1930. This Journal, xci, 390.
SCHNEIDER, E. C. AND D. TRUESDELL. 1922. This Journal, lxiii, 155.
STEWART, G. N. 1911. This Journal, xxviii, 190.
VINCENT, S. AND A. T. CAMERON. 1916. Quart. Journ. Exper. Physiol., ix, 45.
VINCENT, S. AND J. H. THOMPSON. 1928. Journ. Physiol., lxvi, 307.

# THE REGULATION OF RESPIRATION

## A Study of the Correlation of Numerous Factors of Respiratory Control During Altered Pressures of Alveolar Oxygen[1,2]

ROBERT GESELL, HUGO KRUEGER,[3] GRACE GORHAM AND THEODORE BERNTHAL

*From the Department of Physiology, University of Michigan, Ann Arbor*

Received for publication March 17, 1930

Though the probability of correlated changes in activity and acidity of the respiratory center under various conditions is supported by experimental study it is in need of extended analysis. If acidity is the predominant factor of control, more quantitative data on the acid-base equilibrium of the body are needed. If impaired oxidations exert their main effects through changes in fixed and volatile acids, more exact information on the changing content of these acids correlated with changes in oxidations is desirable. On the other hand, if oxidations exert an additional control on respiration independent of acidity, it is essential to study the effects which changes in hydrogen ion concentration may exert on tissue oxidations. With such objectives in mind this series of experiments was performed.[4]

Procedure. Tidal air, pulmonary ventilation, respiratory movements, carbon dioxide and oxygen content of expired air, total elimination of

[1] Preliminary report, This Journal, 1929, xc, 358.

[2] This investigation has been made with the assistance of a grant from the Committee on Therapeutic Research, Council on Pharmacy and Chemistry, American Medical Association.

[3] Submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[4] In an effort to picture the interaction of at least two factors in the control of respiration the possibility of oxidation-reduction phenomena and changes in acidity working hand in hand has been suggested. According to this suggestion not only may inpaired oxidations lead to increased acidity and increased acidity lead to impaired oxidations but both changes may lead to comparable end effects on function. This view is expressed in a very tentative form in an electrochemical hypothesis of control (Gesell, 1926). Since the administration of carbon dioxide as well as impairment of oxidations by hemorrhage, lowered alveolar oxygen and cyanide lead to an increase in reducing metabolites as demonstrated with the manganese dioxide electrode (Gesell and Hertzman, 1928a, b; Hertzman and Gesell, 1927) the net result of synchronous changes in acidity and in oxidation-reduction potentials deserves further consideration. This aspect of the subject, however, must await later analysis

carbon dioxide and total absorption of oxygen, expiratory quotient, lactic acid content of the blood, carbon dioxide capacity of the blood, hydrogen ion concentration of the blood, volume flow of blood, mean arterial blood pressure, oxidative energy, non-oxidative energy, the relation of shortage of oxygen consumption to accumulated lactic acid, the relation of excess oxygen consumption to the disappearance of lactic acid, the relation of total elimination of carbon dioxide to that required by oxidations, the relation of the lactic acid content of the body to the liberation and retention of carbon dioxide, the proportion of fixed acid combining with bicarbonate and the proportion reacting with other buffer systems were studied.

The experiments were done on large heparinized dogs (about 25 kgm. body weight) anesthetised with morphine and urethane. Pneumothorax was established and constant artificial ventilation administered as described in earlier publications. With prolonged constant ventilation with any single gas, changes in composition of the expired air are roughly indicative of changes in the total amount of carbon dioxide expired and total amount of oxygen absorbed. Continuous records of expired oxygen and carbon dioxide, therefore, served as valuable guides to changes in gaseous metabolism during the course of the experiment.

Changes in expired oxygen were recorded with the continuous combustion method previously described (Gesell and McGinty, 1926). Upstroke indicates decreasing absorption of oxygen, when a uniform gaseous mixture is continuously administered. When a gaseous mixture low in oxygen is substituted for room air the downstroke is not indicative of increased absorption, but is due to the changes in the oxygen content of the air. On the other hand, when room air is readministered the comparative heights of the curves before and after administration of low oxygen are again inversely related to the rate of oxygen consumption (see fig. 1).

Changes in expired carbon dioxide were recorded with the katharometer. A carbon dioxide cell such as described by Rosencranz (1927) and manufactured by Leeds and Northrup Company was employed as illustrated in figure 2. Two fine platinum wires connected in a Wheatstone bridge circuit are heated by current passing through the bridge. One of the platinum wires is sealed in a tube, *A*, containing dry air and the other is sealed in a tube, *B*, through which the carbon dioxide containing gas diffuses. Due to the low thermal conductivity of carbon dioxide and the relatively equal thermal conductivity of nitrogen and oxygen the electrical balance of the bridge is greatly disturbed by changes in the carbon dioxide content of the gas and is relatively little disturbed by changes in the proportion of oxygen and nitrogen. The change in resistance required to balance the bridge is thus a fairly accurate index to the change in carbon dioxide content. This changing resistance is mechanically registered on smoked paper by a vertically-writing point attached by thread and pulley to a spindle

on a Kohlrausch wire inserted in the bridge. Upstroke indicates increasing expired carbon dioxide.

The general arrangement for supplying expired air to the carbon dioxide cell is shown in figure 2. An ordinary water aspirator, *N*, draws the sample from the tracheal cannula, *J*. The air passes up the drying column, *K*, which is filled below with calcium chloride for extraction of the bulk of the moisture and above with dehydrite for the extraction of the last traces of moisture. The connection with the drying column is made with a T tube, *L*, which serves as a trap for the condensed moisture. The air then passes through a set of fine copper coils mounted in a constant temperature box held at 35°C. for temperature adjustment. The air then passes through tube *C* of the carbon dioxide cell, also mounted in the constant temperature box, into the 20 liter bottle, *O*, and out through the aspirator. By virtue of the heating effects of the platinum wire mounted in tube *B*, gas circu-

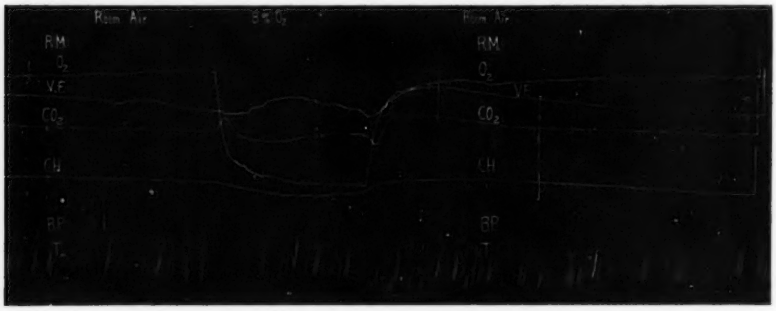



Fig. 1

ates through *B* and *D* rising in *B* and falling in *D*. The rate of circulation in *B* and *D*, is, therefore, within certain limits independent of the rate of aspiration. The period of response to a change in carbon dioxide content of the expired air should, therefore, be constant provided the rate of aspiration is constant. To insure this uniform movement of gas throughout this series of experiments the aspirator was supplied with water at a constant head of pressure by means of an automatically adjusted reservoir. The 20 liter bottle was inserted in the air circuit as an air cushion to eliminate momentary changes in E. M. F. due to sharp irregularities of the pump.

This arrangement for following changes in expired carbon dioxide was used in all of the experiments where room air was continuously administered. When artificial gaseous mixtures are substituted for room air the method as described is no longer suited to our needs. For example, the administration of a gaseous mixture low in oxygen following on room air

alters the proportion of oxygen to nitrogen sufficiently to mask the thermal conductivity effects of carbon dioxide. In addition these artificial gaseous mixtures contain impurities not found in room air. To automatically correct for any differences in gaseous composition other than expired carbon dioxide a double carbon dioxide cell was substituted for the single cell. In this arrangement as shown in the left side of the constant temperature box, cell $A'$ is no longer sealed, but is put in shunt with the air current like cell $B'$. The expired air is drawn this time through tube $C$, then through column $H$ containing a mixture of ascarite and dehydrite. The ascarite absorbs the carbon dioxide and the dehydrite absorbs the moisture formed

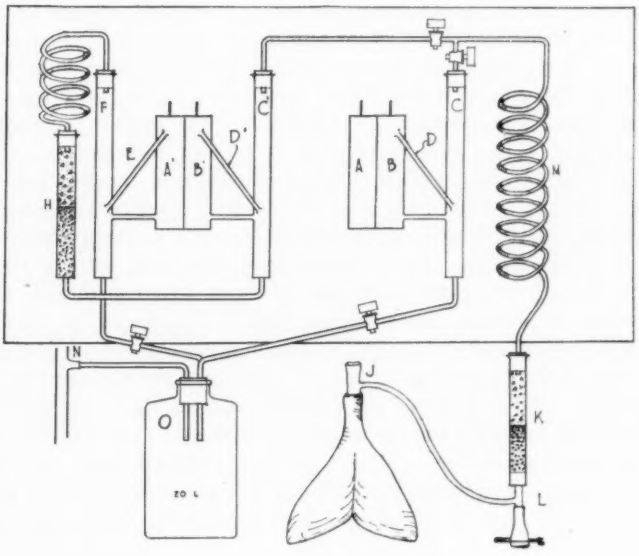



Fig. 2

in the chemical reaction. The gas then passes through a second copper tube for temperature adjustment and then through $F$ to tube $A$. The only difference between the gases surrounding wires $A$ and $B$ is the concentration of carbon dioxide. However, on changing from gas 1 to gas 2 wire $B$ may be temporarily surrounded by gas 2 while wire $A$ is still surrounded by gas 1. Consequently on changing from one gas to another there is a temporary deflection lasting about 70 seconds which cannot be used as an index to carbon dioxide elimination. After gas 1 has been completely displaced the disturbance in electrical balance is due to the difference in the carbon dioxide content of the gases in tubes $A'$ and $B'$. The

record (fig. 2) shows an initial upward deflection on changing from room air to a low oxygen mixture and an initial downward swing on changing to room air again.

While the continuous methods have not been refined for purposes of quantitative determination of gaseous exchange they have been invaluable in following the course of experiments. With their aid we were enabled to see changes in gaseous metabolism at the time of their occurrence and, thereby, direct the course of the experiment more effectively. The curves described by these methods have told us more accurately the duration of the changes in gaseous metabolism and have indicated the optimum moment for blood and gas sampling. Gas samples of expired air were thus taken at advantageous times with 50 cc. luer glass syringes from a gas reservoir of several hundred cubic centimeter capacity. Before reaching this reservoir the air passes down a rubber tube one meter long, through a rotating stopcock of the artificial respiration pump, then up another vertical tube 1 meter long. The capacity of the loop is approximately 700 cc. The air is in motion one-half of the time as the lungs are collapsing and the rotating cock is open. To avoid effects of possible incomplete mixing of the expired air at the reservoir, the fifty cubic centimeter samples were drawn with a steady continuous pull over a uniform period of ten strokes of the pump. The samples were analyzed with a Haldane-Henderson analyzer. The percentage of expired carbon dioxide and expired oxygen are entered on each graph (figs. 3, 4, 5, 6, and 7). The upper number is expired carbon dioxide, second number expired oxygen and the third number the "expiratory quotient" and the lower number that of the gas sample. The location of the decimal point indicates the moment of sampling with reference to time which is registered in one and five minute intervals on the abscissas. Where the gas analyses could not be conveniently placed at the proper point on the graph an arrow is inserted pointing to the time of sampling. Zero time indicates the beginning of observations.

Oxygen consumption and carbon dioxide elimination in cubic centimeter (standard conditions) per kilogram of body weight per hour are computed for all points at which gas samples were taken. Oxygen consumed equals volume of oxygen inspired per minute minus volume of oxygen expired per minute. Oxygen consumed in cubic centimeter per minute per kilogram, therefore, equals per cent $O_2$ in inspired air times cubic centimeter of inspired air per minute minus per cent $O_2$ in expired air times cubic centimeter of expired air per minute over the weight of the animal in kilogram. Similarly the carbon dioxide eliminated in cubic centimeter per kilogram minute equals per cent of $CO_2$ in expired air times cubic centimeter of expired air minus per cent of $CO_2$ in inspired air times cubic centimeter of inspired air over the weight of the animal in kilogram. The volume

of inspired air delivered to the animal was measured directly with a gas meter and converted to standard conditions. The volume of the expired air reduced to standard condition was then computed.

$Vo$ expired $= \frac{\text{per cent } N_2 \text{ in expired air}}{\text{per cent } N_2 \text{ in inspired air}}$ times $Vo$ of inspired air. The rates of oxygen consumption and carbon dioxide elimination were plotted on the ordinates against time on the abscissas. The subtended area, therefore, gives the volume of gaseous exchange per period of time. The curves were more accurately shaped with the use of the original continuous records. One centimeter on the ordinates of the original graph before photographic reduction equals 20 cc. of gas per kilogram hour and on the abscissas 1 cm. equals 1 minute. One square centimeter therefore represents 20 cc. of gaseous exchange.

Difficulty in identifying the curves at points of crossing is avoided by consulting the "expiratory quotients" which tell the relative position of the two curves. For expiratory quotients below 1.00 the oxygen consumption curve is higher and for quotients above 1.00 the carbon dioxide elimination curve is higher. By continuing the preadministration gradient of oxygen consumption the underlying and overlying areas give an indication of the shortage of oxidations under normal and of the excess above normal for the corresponding period of time. The same information with respect to carbon dioxide elimination is obtained by extending the carbon dioxide elimination gradient.

A 13 cc. blood sample, usually arterial, drawn by syringe from the manganese dioxide electrode vessel served for the determination of blood pH, blood carbon dioxide capacity and blood lactic acid. The time of sampling is not always clearly indicated in the graph but can be found with the use of the tables. After each sample an equal amount of saline solution was injected intravenously to maintain the circulation. On drawing the blood sample, 4 cc. were delivered into 500 cc. separatory funnels filled with alveolar air. This was used for carbon dioxide capacity determinations made with the Van Slyke method (Van Slyke and Neill, 1924, and Harrington and Van Slyke, 1924). The next cubic centimeter of blood was delivered into 1 cc. blood sample tubes for pH determinations made with the capillary quinhydrone electrode according to the procedure described by one of us (Gesell, 1919). The remaining blood was used for lactic acid determinations by the Friedemann, Cotonio and Shaffer method (1927).[5]

Blood lactic acid in milligram per 100 cc. of blood is plotted on the ordi-

[5] From two to three determinations of carbon dioxide capacity, three to six of hydrogen ion concentration and three to five of lactic acid content were made depending on how well the determinations checked.

nates against time on the abscissas. Blood carbon dioxide capacity is plotted in volumes per cent as an inverted curve to facilitate the comparison of carbon dioxide and lactic acid content of the blood. The scale of plotting is such that one volume per cent is equivalent to 4 mgm. of lactic acid—which represents the volumetric liberation of carbon dioxide when lactic acid reacts completely with sodium bicarbonate. Thus parallel curves indicate equivalent changes, but in opposite direction, of lactic acid and carbon dioxide content of the blood. The blood pH curve is also inverted.

Since variations in oxidations lead to interrelated changes in the liberation and disappearance of lactic acid and liberation and retention of carbon dioxide we have studied this relation in an approximate way with the use of the "expiratory quotient." Maintaining a constant pulmonary ventilation, as in our earlier experiments on gaseous exchange, blowing off of carbon dioxide due to increased ventilation is avoided. The ratio of elimination of carbon dioxide to absorption of oxygen, therefore, becomes considerably more significant in the interpretation of gaseous metabolism and acid-base changes. It is assumed that, other things remaining constant, a liberation of carbon dioxide in the blood and tissues by an accumulation of lactic acid during a period of impaired oxidations would alone produce a disproportionate elimination of carbon dioxide and show itself in the expiratory quotient. Similarly if accumulated lactic acid disappears by non-oxidative as well as oxidative processes when oxidations return with readministration of room air the expiratiory quotient should indicate a retention of carbon dioxide. That such changes in the expiratory quotient occur with the lactic acid shift during cyanide poisoning and recovery has been demonstrated by McGinty and Gesell (1927). Changes in the expiratory quotient are, therefore, worthy of consideration in connection with the more complete picture of respiratory disturbances which we now present.

The relation of carbon dioxide elimination to lactic acid metabolism is also followed in these experiments by comparing the carbon dioxide equivalent of changing lactic acid content of the body with the computed elimination of non-oxidative carbon dioxide. As an index to the total lactic acid content of the body we have used the concentration of lactic acid in the blood assuming equal distribution between the tissues and the blood. This assumption may be somewhat incorrect, for the concentration of lactic acid is probably higher in the tissues than in the blood during conditions of equilibrium and during periods of rapid formation and possibly lower in the tissues than in the blood during periods of rapid disappearance, but it must serve temporarily as a means of approximation. On this basis the accumulation of lactic acid in milligram per kilogram of body tissue per hour is calculated for each period between blood samples. The equivalent amount of carbon dioxide liberated with complete reaction

with bicarbonate is computed and plotted in block form in cubic centimeter per kilogram hour as $CO_2 \infty$ of L.A. The areas above the base line indicate the theoretical maximum liberation of carbon dioxide from accumulated lactic acid and the areas below the base line the theoretical maximum retention of carbon dioxide, assuming complete non-oxidative removal of the lactate anion leaving sodium free to combine with carbon dioxide to form sodium bicarbonate.

Assuming that carbon dioxide liberated by lactic acid is freely eliminated from the blood as it passes through the lungs these areas compared with corresponding areas of elimination of carbon dioxide of non-oxidative origin give a rough index of the effectiveness of lactic acid in combining with sodium bicarbonate and a somewhat clearer picture of the acid base equilibrium of the body. Non-oxidative carbon dioxide was computed by multiplying the oxygen consumption by the expiratory quotient under normal conditions and subtracting this amount from the total observed carbon dioxide elimination. This value is plotted as $CO_2$ (non-oxidative).

Energy relations are also indicated in the tables and graph. Energy of oxidative origin is computed with the use of the curve of oxygen consumption, the expiratory quotient, and tables of equivalents of oxygen in calories. The results are presented in small calories per kilogram hour. Where the lactic acid content of the blood remains constant it is assumed that the oxidative energy represents the total energy available which is plotted as a solid black line. Where lactic acid is changing in amount the energy exchange associated with the lactic acid shift of anaerobic metabolism is taken into account in establishing the total energy curve. When lactic acid is increasing in amount the equivalent anaerobic energy is added to the oxidative energy. When lactic acid is disappearing the equivalent energy loss is subtracted from the oxidative energy. The energy change due to lactic acid shift is also plotted in block form for every period between blood samples. The square area above or below the base line respresents the energy equivalent. The areas above represent the energy derived from liberation of lactic acid and the areas below the energy lost by its disappearance, assuming that all of the lactic acid disappears by non-oxidative processes. The quantitative relation of shortage and excess of oxygen consumption to accumulation and disappearance of lactic acid is not clearly apparent from the graphs. This relation is computed and presented in the body of the paper. Here again an equal distribution of lactic acid in blood and tissues is assumed.

Results. Before analyzing the effects of administration of gaseous mixtures low in oxygen, attention is called to the general deteriorating effects of heparin and artificial ventilation. This is seen in the progressive decrease in gaseous metabolism and mean blood pressure. If this deterioration is extensive during the preliminary administration of room air

and of low oxygen and of readministration of room air it is essential to take this factor into account to get a clear picture of the reaction of the animal to the changed conditions. The degree of deterioration is indicated by the gradient of the curves of oxygen consumption and carbon dioxide elimination. Thus by extending the initial gradient of the curves through the subsequent periods of administration of low oxygen and of room air a rough correction for this deterioration with respect to gaseous exchange may be made. The areas below and above these gradients represent the deficient and excess exchange respectively. In experiment 1 circulatory and metabolic deterioration were relatively small and the correction obtained by extending the gradients is correspondingly small. In this experiment, as in many others, isotonic saline solution was injected to counteract progressive deterioration. The supporting effects of such injection are quite apparent in the graph.

Experiment 1 begins with prolonged administration of room air which is followed by administration of a 9.19 per cent oxygen mixture in nitrogen lasting 15 minutes 32 seconds and a subsequent readministration of room air. During the initial administration of room air the rate of oxygen consumption and carbon dioxide elimination remained approximately constant. With lowered alveolar oxygen the absorption fell sharply from 384 cc. per kgm. hour to 228 cc. This lowered rate then remained nearly constant up to the readministration of room air when the maximum rate of 452 cc. was quickly attained. The rate then fell off gradually and within sixteen minutes reached the preadministration level. The areas below and above the extended gradient show a shortage of oxygen absorption during the administration of low oxygen and an excess absorption during the readministration of room air. The compensatory consumption falls considerably short of the preceding deficiency. The effects of lowered alveolar oxygen and readministration of room air on carbon dioxide elimination are decidedly different. While the elimination was diminished during lowered alveolar oxygen the reduction (from 284 to 275 cc. per kgm. hour) was very small as compared with the reduction of oxygen consumption and again though the elimination of carbon dioxide was increased on readministration of room air the increase was very small when compared with the associated increase in oxygen consumption. Corresponding to these alterations in gaseous exchange the expiratory quotient increased from 0.75 and 0.74 during the initial administration of room air to 1.20 and 1.19 during lowered alveolar oxygen. On readministration of room air it dropped suddenly to 0.65 and then slowly returned to preadministration values. These results are in accord with the results of Davis and Slater (1926, 1927, 1928) on aerobic and anaerobic metabolism of the cockroach and earthworm.

Apparently some mechanism other than the direct formation of carbon

dioxide from oxidations was at work determining the amount of carbon dioxide eliminated through the lungs. Since pulmonary ventilation is eliminated the factors involved may be confined primarily to chemical changes in the body and to changes in volume flow of blood. Looking to the blood for the appearance of lactic acid it will be seen that lactic acid is increased from 27.6 mgm. per 100 cc. of blood to 45.4 mgm. during the period of impaired oxidations. The reduction in carbon dioxide capacity of the blood indicates that lactic acid was effective in displacing carbon dioxide. Compare the curves of blood carbon dioxide capacity and blood

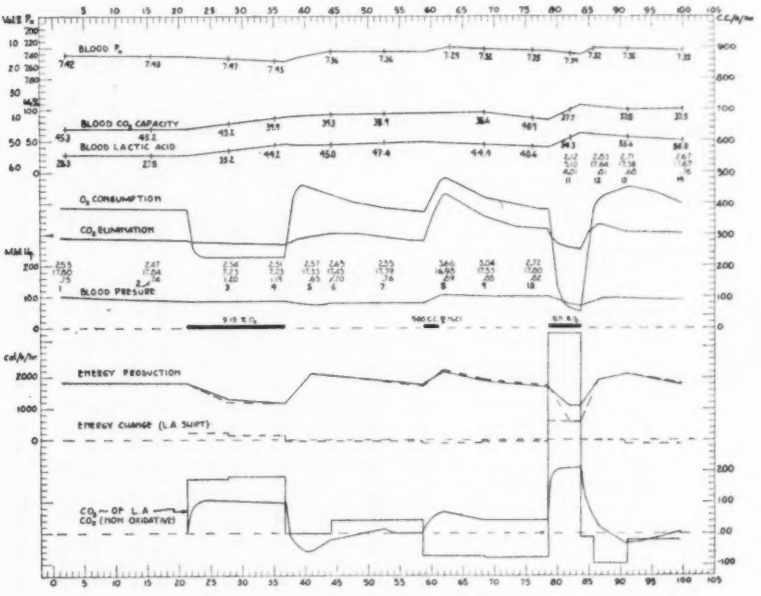



Fig. 3. Experiment 1

lactic acid. For further analysis of carbon dioxide elimination the carbon dioxide equivalent of lactic acid which has accumulated may be consulted as well. This is plotted in block form in cubic centimeter per kilogram hour. The curve shows changes in rate of accumulation or of disappearance of lactic acid more clearly than the blood lactic acid curve which is plotted in smaller scale. The elimination of non-oxidative carbon dioxide is plotted on the same base line. Ignoring the possibility that the rate of elimination of carbon dioxide already formed and stored in the body remains unaltered, a comparison of the areas confined between the base line and the curves of $CO_2 \infty$ of L.A. and $CO_2$ (non-oxidative) gives a rough

TABLE 1

*Experiment 1*

| OBSERVATION | PROCEDURE | TIME | PER CENT OF EXPIRED $CO_2$ | PER CENT OF EXPIRED $O_2$ | EXPIRATORY QUOTIENT | $O_2$ CONSUMPTION IN CC. PER KGM. HR. | $CO_2$ ELIMINATION IN CC. PER KGM. HR. | $CO_2$ ELIMINATION FROM OXIDATIONS BASED ON E.Q. IN CC. PER KGM. HR. | DIFFERENCE BETWEEN ACTUAL AND THEORETICAL $CO_2$ ELIMINATION | $CO_2$ EQUIVALENT OF CHANGE OF LACTIC ACID | LACTIC ACID IN MGM. PER 100 CC. ARTERIAL BLOOD | $CO_2$ CAPACITY OF ARTERIAL BLOOD IN VOL. PER CENT | pH OF ARTERIAL BLOOD | MEAN BLOOD PRESSURE | OXIDATIVE ENERGY IN SMALL CALORIES PER KGM. HR. | NON-OXIDATIVE ENERGY IN SMALL CALORIES PER KGM. HR. | OXIDATIVE + NON-OXIDATIVE ENERGY IN SMALL CALORIES PER KGM. HR. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 |
| 1 | G. S. no. 1 | 1′ 16″ | 2.53 | 17.80 | 0.75 | 387.6 | 291.6 | 292 | | | | | | 102 | 1,838 | | |
| 2 | A. S. no. 1 | 2′ 0″ | | | | | | | | | 28.3 | 45.27 | 7.42 | | | | |
| 3 | G. S. no. 2 | 15′ 23″ | 2.47 | 17.84 | 0.74 | 384.0 | 284.5 | 289 | −5 | | | | | 90 | 1,823 | −7 | 1,816 |
| 4 | A. S. no. 2 | 15′ 27″ | | | | | | | | | 27.8 | 45.22 | 7.43 | | | | |
| 5 | 9.19% $O_2$, 0.02% $CO_2$ | 21′ 15″ | | | | | | | | | c 27.6 | | | 86 | | | |
| 6 | G. S. no. 3 | 27′ 38″ | 2.34 | 7.23 | 1.20 | 228.8 | 275 | 172 | +103 | +174 | | | | 86 | 1,085 | +211 | 1,296 |
| 7 | A. S. no. 3 | 27′ 48″ | | | | | | | | | 35.2 | 43.15 | 7.47 | | | | |
| 8 | G. S. no. 4 | 34′ 58″ | 2.31 | 7.23 | 1.19 | 228.3 | 271.4 | 172 | +100 | +183 | | | | 86 | 1,083 | +224 | 1,307 |
| 9 | A. S. no. 4 | 35′ 10″ | | | | | | | | | 44.2 | 39.88 | 7.45 | | | | |
| 10 | Room Air | 36′ 47″ | | | | | | | | | c 46.0 | | | | | | |
| 11 | G. S. no. 5 | 40′ 43″ | 2.57 | 17.33 | 0.65 | 451.9 | 293.1 | 340 | −47 | −20 | | | | 76 | 2,140 | −25 | 2,115 |
| 12 | G. S. no. 6 | 43′ 52″ | 2.65 | 17.45 | 0.70 | 432.1 | 303.2 | 325 | −22 | −20 | | | | 78 | 2,050 | −25 | 2,025 |
| 13 | A. S. no. 5 | 44′ 3″ | | | | | | | | | 45.0 | 39.28 | 7.36 | | | | |
| 14 | G. S. no. 7 | 52′ 27″ | 2.55 | 17.79 | 0.76 | 386.8 | 291.8 | 277 | +15 | +42 | | | | 80 | 1,835 | +51 | 1,886 |
| 15 | A. S. no. 6 | 52′ 37″ | | | | | | | | | 47.4 | 38.93 | 7.36 | | | | |
| 16 | 500 cc. Saline | 58′ 53″ | | | | | | | | | c 49.0 | | | 80 | | | |
| 17 | G. S. no. 8 | 62′ 0″ | 3.56 | 16.98 | 0.89 | 482.1 | 430.9 | 363 | +68 | −71 | | | | 106 | 2,282 | −87 | 2,195 |

| | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 18 | A. S. no. 6A | 62′ 58″ | | | | | | | | | | | 7.29 | | | | |
| 19 | G. S. no. 9 | 68′ 27″ | 3.04 | 17.53 | 0.85 | 418.6 | 356.8 | 315 | +42 | −71 | | | | 100 | 1,986 | −87 | 1,899 |
| 20 | A. S. no. 7 | 68′ 31″ | | | | | | | | | 44.4 | 38.62 | 7.32 | | | | |
| 21 | G. S. no. 10 | 75′ 50″ | 2.72 | 17.80 | 0.82 | 386.9 | 318.5 | 277 | +42 | −76 | | | | 100 | 1,835 | −92 | 1,743 |
| 22 | A. S. no. 8 | 76′ 3″ | | | | | | | | | 40.6 | 40.86 | 7.35 | | | | |
| 23 | 5.715% $O_2$, 0.01% $CO_2$ | 78′ 38″ | | | | | | | | | c 39.3 | | | 100 | | | |
| 23A | G. S. no. 11 | 81′ 57″ | 2.12 | 5.10 | 4.01 | 63.8 | 255.7 | 48 | +208 | +642 | | | | 76 | 306 | +781 | +1,087 |
| 24 | A. S. no. 9 | 82′ 8″ | | | | | | | | | 54.3 | 37.74 | 7.39 | | | | |
| 24A | Room Air | 83′ 48″ | | | | | | | | | c 61.26 | | | | | | |
| 25 | G. S. no. 12 | 86′ 30″ | 2.83 | 17.64 | 0.81 | 408.0 | 331.4 | 307 | +24 | +22 | | | | 90 | 1,936 | −14 | 1,922 |
| 25A | A. S. no. 9A | 85′ 45″ | | | | | | | | | | | 7.32 | | | | |
| 26 | G. S. no. 13 | 90′ 50″ | 2.71 | 17.30 | 0.68 | 449.1 | 307.1 | 338 | −31 | +22 | | | | 94 | 2,131 | −14 | 2,117 |
| 27 | A. S. no. 10 | 91′ 8″ | | | | | | | | | 55.6 | 37.81 | 7.32 | | | | |
| 28 | G. S. no. 14 | 99′ 43″ | 2.67 | 17.67 | 0.76 | 397.3 | 303.2 | 299 | +4 | −95 | | | | 88 | 1,885 | −116 | 1,769 |
| 29 | A. S. no. 11 | 99′ 57″ | | | | | | | | | 50.0 | 37.51 | 7.35 | | | | |

idea of the proportion of lactic acid combining with bicarbonate. This is expressed more accurately by the quotient of $CO_2$ (non-oxidative) over $CO_2\infty$ of L.A. which will be referred to hereafter as the carbon dioxide index. The graph indicates a rapid accumulation of lactic acid associated with a rapid increase in elimination of non-oxidative carbon dioxide. For the period intervening between the administration of low oxygen and gas sample 3 the carbon dioxide index was 0.61 (table 6); for the period between gas samples 3 and 4 it was 0.55; and for the period between gas sample 4 and the readministration of room air it was 0.61. The average index for the entire period of low oxygen was 0.58. Between the readministration of room air and gas sample 5 there was a retention of carbon dioxide amounting to 1.55 cc. per kgm. and a disappearance of lactic acid equivalent to 1.25 cc. The index of retention of carbon dioxide was 1.24. From gas sample 5 to 6 the index of retention has risen to 1.42. Between gas samples 6 and 7 the carbon dioxide index has a negative value of −0.12. Carbon dioxide is still being retained although the lactic acid content of the blood is increasing. From gas sample 7 to administration of saline solution there was an excess carbon dioxide eliminated again above that required by oxidations. Lactic acid simultaneously increased. The index, therefore, becomes positive again. From room air to injection of saline solution the index was −0.53. The figure which is more significant is the index covering the more inclusive period beginning with the administration of low oxygen and ending at the onset of saline injection. According to the index of this period 43 per cent of the lactic acid which accumulated combined with bicarbonate.

Following next the changes in hydrogen ion concentration of the arterial blood it will be seen that the pH remained virtually constant during the initial administration of room air −7.42 and 7.43. On administration of 9.19 per cent oxygen the pH increased to 7.47 and then swung slightly acid to 7.45. With readministration of room air there was a sharp increase in acidity to pH 7.36 which was then fairly well sustained until the injection of saline solution. It is interesting to note that the arterial blood turned alkaline during the administration of 9.19 per cent oxygen mixture for blood lactic acid increased, blood carbon dioxide capacity diminished and the output of carbon dioxide and the percentage of expired carbon dioxide was very little diminished. The hemoglobin, however, was of course more reduced which would contribute an alkaline effect. On readministration of room air the acid change is more readily understood for all of the significant factors were working in the acid direction. Lactic acid continued to increase, blood carbon dioxide capacity continued to diminish, the carbon dioxide pressure of the blood was increased and the blood was more highly oxidized.

At 58 minutes 53 seconds 500 cc. isotonic saline solution were injected.

The details of the effects of such injection will be taken up in another paper dealing with changes in blood volume. For present purposes only the general effects need be noted—the increase in oxygen consumption, in carbon dioxide elimination, expiratory quotient and carbon dioxide capacity of the blood and a decrease in blood lactic acid and hydrogen ion concentration.

At 78 minutes 38 seconds administration of 5.71 per cent oxygen was superimposed upon these new conditions and at 83 minutes 48 seconds room air was readministered. During lowered alveolar oxygen, oxygen absorption was enormously diminished—falling from 386.9 cc. per kgm. hour to 63.8 cc. This reduction was considerably greater than that during the administration of 9.19 per cent oxygen. On readministration of room air the oxygen absorption rose to a maximum of 449.1 cc. and gradually dropped to 353.3 cc. at 126 minutes 15 seconds. The absolute fall in carbon dioxide elimination was also considerably greater during lowered alveolar oxygen than in the preceding administration of 9.19 per cent oxygen yet in relation to the rate of oxygen absorption carbon dioxide elimination was enormously increased for the expiratory quotient rose from 0.82 to 4.01. With readministration of room air carbon dioxide elimination temporarily increased above normal as in the preceding curve but the expiratory quotient also fell to a subnormal level of 0.68. Though it rose again to 0.76 this is still 0.06 below the expiratory quotient just before the administration of 5.71 per cent oxygen. The results definitely indicate a retention of carbon dioxide similar to that which occurred on the previous readministration of room air. Looking again for the explanation of the changes in the expiratory quotient we find an exceedingly rapid accumulation of lactic acid in the blood during the period of anoxemia and a fairly rapid removal during the subsequent period of hyper-oxidations. Assuming a non-oxidative as well as oxidative removal of lactic acid the retention of carbon dioxide is explained. A comparison of the curve of non-oxidative carbon dioxide with the curve of oxygen consumption explains graphically the accompanying changes in the expiratory quotient. The carbon dioxide index between the administration of 5.71 per cent oxygen and gas sample 11 was 0.24, between gas sample 11 and administration of room air 0.33 and for the total period between the administration of 5.71 per cent oxygen and gas sample 14 it was 0.68. The parallel course of blood lactic acid and blood carbon dioxide capacity is quite striking. Again it is worthy of note that the blood turns alkaline during the administration of low oxygen (from pH 7.35 to 7.39) despite the accumulation of lactic acid and the disappearance of bicarbonate in the blood, to turn momentarily acid on readministration of room air.

It seemed of interest to attempt an approximate determination of the energy balance of the body during impaired oxidations and later recovery.

Two kinds of energy are considered—the energy derived from oxidative processes or aerobic energy and the energy derived from non-oxidative processes or anaerobic energy. The oxidative energy is computed with the aid of the oxygen absorption curve, the expiratory quotient and a table of caloric equivalents (Lusk, 1923). As source of change in the non-oxidative energy we have considered only the lactic acid shift. The heat evolved by the conversion of glycogen into lactic acid plus the heat of dilution and neutralization of the acid which is formed is added to the oxidative energy. The absorption of energy on reconversion of lactic acid into glycogen is subtracted from the oxidative energy. Thus when glycogen gives rise to one gram of lactic acid 180 calories of heat are liberated. Adding to this the heat of dilution and neutralization of the acid the total dissipation of energy is equivalent to 304 calories (Meyerhof, 1925). This energy if available for the general use of the living cell is significant. To what extent either portion is available is in no way indicated by our results. We have, however, plotted two energy curves, the heavier including the complete anaerobic energy and the broken curve including the first portion only.

On the whole the total energy dissipated during anoxemia was less than that dissipated by the animal during normal oxidations. The increase in anaerobic energy failed to keep pace with the diminution in aerobic energy. Obviously a large shortage of oxygen consumption and a small formation of lactic acid is accompanied by a relatively small dissipation of energy and a small shortage of oxygen consumption and a large formation of lactic acid is accompanied by a relatively large dissipation of energy. Thus by dividing the oxygen shortage in cubic millimeters during anoxemia by the amount of lactic acid in milligrams which accumulates a quotient is obtained indicating the general level of the total energy dissipation. By computing the ratio of the change in oxygen shortage to the change in lactic acid formation which is unaccompanied by a change in energy dissipation we get a significant figure for general purposes of comparison. This may be computed for any expiratory quotient. When the expiratory quotient is 0.80, which is approximately the average for our experiments on the dog, oxidations using one liter of oxygen provide 4,800 calories. Since the formation of one gram of lactic acid provides 180 or 304 calories depending on whether or not the heat of dilution and neutralization is included it will require the liberation of 26.1 or 16 grams of lactic acid respectively to provide the same energy. The oxygen-lactic acid index for complete restoration of energy lost from impaired oxidations would be $\frac{1000}{26.6}$ or $\frac{1000}{16}$. In the first instance the index is 37.5 and in the second 63.4. Therefore, if the expiratory quotient is 0.80 and the oxygen-lactic acid index is less than 63.4 the curve of energy dissipation should rise, that is, the energy released by the formation of lactic acid is more than the

thermodynamic equivalent of the oxygen shortage. If the ratio is greater the curve should fall. Slight exceptions to this statement will be pointed out where they occur. They are due to our method of plotting—of extending the curve beyond points of blood and gas sampling. This involves errors if the blood and gas samples are too widely separated.

The relation of variations in oxidation to variations in the body content of lactic acid (as indicated by the blood) for the six experiments of this series are grouped in tables 6 and 7. The oxygen shortage below the initial normal level is indicated by the negative values in column 1 and the excess oxygen consumption above the initial level by the positive values. Column 2 shows the corresponding changes in lactic acid. The ratio of oxygen shortage over lactic acid increase is tabulated in column 3 while the ratio of the oxygen excess to decrease in lactic acid is given in the fourth column. Not infrequently and for reasons that are not yet entirely clear, lactic acid may diminish in the blood though oxidations are below normal and also the reverse, lactic acid may increase in amount though oxidations are above the normal rate. The oxygen shortage or excess and the lactic acid decrease or increase under these conditions are included but without the corresponding ratios.

In experiment 1 between the administration of 9.19 per cent oxygen and gas sample 3 the oxygen-lactic acid index was 222; between gas samples 3 and 4 it was 208; between gas sample 4 and the administration of room air it was 238. The average figure for the entire period of anoxemia was 217. Since the oxidation of one gram of lactic acid requires 740 cc. of oxygen, for every shortage of 740 cc. of oxygen there were formed 740 over 217 or 3.45 grams of lactic acid. (This agrees with ratios established by Meyerhof and Hill for isolated muscle.) On readministration of room air there was excess oxygen consumption accompanied first by a moderate removal of lactic acid, between the administration of room air and gas sample 5. For this period the oxygen-lactic acid ratio was 366 indicating that for every 740 cc. excess of oxygen absorption 2.02 grams of lactic acid were removed, agreeing with a retention of carbon dioxide indicated by the lowering of the expiratory quotient. From gas sample 5 to gas sample 6 the lactic acid disappeared at a very slow rate. The oxygen-lactic acid ratio was 602, which means a very small non-oxidative removal of lactic acid, a total removal 1.23 grams for every 740 cc. excess oxygen absorption. Of this 0.23 gram was removed by non-oxidative processes. This high oxygen-lactic acid ratio could be explained along at least four different lines—the lactic acid may leave the tissues very slowly during the rapid accumulation of lactic acid in the cells and, therefore, continue to leave the tissues even though it is disappearing within the tissues with later improvement in oxidations; it may leave the blood slowly during the period of recovery when lactic acid is disappearing within the cells; there may be

a relatively larger carbohydrate metabolism; or it may mean that the mechanism for restoring lactic acid to its precursor state has been impaired by the period of anoxemia. Clearly all four factors could be operating simultaneously. The oxygen-lactic acid index over the entire period between administration of low oxygen to intravenous injection of saline was 148 indicating that for the entire period for every shortage of 740 cc. of oxygen absorption 5 grams of lactic acid were liberated. This means that the loss of oxidative energy was compensated to the extent of 43 per cent by lactic acid formation.

In the second administration of low oxygen (5.71 per cent) there was a very large shortage in oxygen consumption. This, however, was accompanied by a large accumulation of lactic acid in the blood. The oxygen-lactic acid ratio from the second low oxygen administration to gas sample 11 was 85 and from gas sample 11 to room air it was 127. For the total period of anoxemia the index was 100—that is, 7.4 grams of lactic acid were formed for every shortage of 740 cc. of oxygen. During the very first part of the period of recovery our methods indicate a removal of lactic acid. The later coefficients, however, are more accurate. Taking the oxygen consumption just prior to the second administration of low oxygen as the basis of comparison, the excess oxygen consumption between gas samples 12 and 13 and 13 and 14 was relatively small and the removal of lactic acid relatively large. The oxygen lactic acid quotient of removal for these two periods was 120 and 135 respectively. For the more inclusive period from room air to gas sample 14 the index of removal was 55. The still more inclusive period from the second low oxygen administration to gas sample 14 during which there is a shortage of oxygen and an accumulation of lactic acid the lactic acid quotient of accumulation was 146. Again only 43 per cent of the energy lost from oxidations was supplied by lactic acid formation. The drop in the energy curve during anoxemia is thus explained.

Experiment 2 shows the effects of two administrations of 9.77 per cent oxygen mixtures for 13 minutes 17 seconds and 11 minutes 42 seconds respectively. Between administrations there was an intervening period of 39 minutes 16 seconds with an injection of 850 cc. of saline solution at 50 minutes 17 seconds. Preceding the first administration of low oxygen the rate of oxygen absorption and carbon dioxide elimination remained virtually constant at 298 cc. per kgm. hour. The expiratory quotients were 0.79 and 0.78. At 22 minutes 23 seconds the low oxygen mixture was administered. Oxygen absorption diminished and carbon dioxide elimination increased with the resulting changes in expiratory quotient to 1.13, 1.13, and 1.07 at gas samples 3, 4 and 5 respectively. Associated with the increase in the expiratory quotient there was an increase in blood lactic acid and carotid flow of blood. At 35 minutes 40 seconds room air was

readministered. Oxygen absorption increased and carbon dioxide elimination decreased. Extension of the oxygen consumption curve shows excess of oxygen absorption above normal to be considerably less than the shortage of absorption during the anoxemia. During the total period of anoxemia and recovery there has, therefore, been a shortage of oxygen absorption. This is associated with a final accumulation of lactic acid. Extending the carbon dioxide elimination curve, it is clear that during the period of recovery the total elimination of carbon dioxide is slightly above normal. There has, therefore, been an excess elimination of carbon dioxide

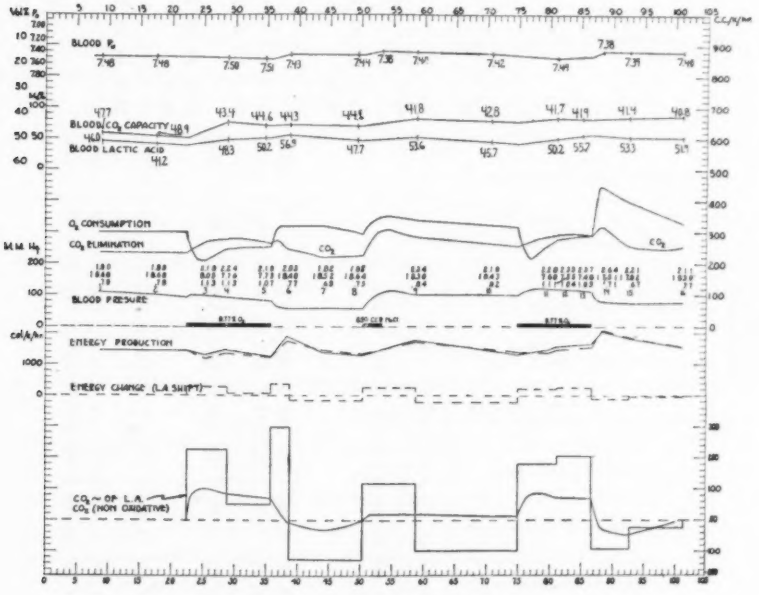



Fig. 4. Experiment 2

during the combined periods. Nevertheless, the expiratory quotients for the period of recovery indicate a retention of carbon dioxide which agrees with the results of experiment one. Over the combined period of anoxemia and recovery there has been a total increase in lactic acid which led to liberation of free carbon dioxide bringing carbon dioxide elimination above the normal level and during recovery there apparently has been a non-oxidative removal of lactic acid which would permit an absolute reduction in carbon dioxide elimination even though oxidation simultaneously increased. The decreased volume flow of blood on readministration of room air would be another factor contributing towards retention of carbon dioxide.

TABLE 2

*Experiment 2*

| OBSERVATION | PROCEDURE | TIME | PER CENT OF EXPIRED $CO_2$ | PER CENT OF EXPIRED $O_2$ | EXPIRATORY QUOTIENT | $O_2$ CONSUMPTION IN CC. PER KGM. HR. | $CO_2$ ELIMINATION IN CC. PER KGM. HR. | $CO_2$ ELIMINATION FROM OXIDATIONS BASED ON E.Q. IN CC. PER KGM. HR. | DIFFERENCE BETWEEN ACTUAL AND THEORETICAL $CO_2$ ELIMINATION | $CO_2$ EQUIVALENT OF CHANGE OF LACTIC ACID | LACTIC ACID IN MGM. PER 100 CC. ARTERIAL BLOOD | $CO_2$ CAPACITY OF ARTERIAL BLOOD IN VOL. PER CENT | pH OF ARTERIAL BLOOD | MEAN BLOOD PRESSURE | OXIDATIVE ENERGY IN SMALL CALORIES PER KGM. HR. | NON-OXIDATIVE ENERGY IN SMALL CALORIES PER KGM. HR. | OXIDATIVE + NON-OXIDATIVE ENERGY IN SMALL CALORIES PER KGM. HR. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 |
| 1 | G. S. no. 1 | 8′ 40″ | 1.90 | 18.68 | 0.79 | 298.2 | 234.6 | | | | | | | 108 | 1,425 | | |
| 2 | A. S. no. 1 | 8′ 53″ | | | | | | | | | 46.0 | 47.72 | 7.48 | | | | |
| 3 | G. S. no. 2 | 17′ 31″ | 1.89 | 18.68 | 0.78 | 298.8 | 233.3 | 235 | | | | | | 100 | 1,427 | −103 | |
| 4 | A. S. no. 2 | 17′ 31″ | | | | | | | | | 41.2 | 48.90 | 7.48 | | | | |
| 5 | 9.774% $O_2$, 0.02% $CO_2$ | 22′ 23″ | | | | | | | | | c 38.6 | | | 92 | | | |
| 6 | G. S. no. 3 | 25′ 20″ | 2.18 | 8.05 | 1.13 | 209.6 | 268.4 | 165 | | +226 | | | | 97 | 1,000 | +275 | 1,275 |
| 7 | G. S. no. 4 | 28′ 35″ | 2.24 | 7.76 | 1.13 | 244.8 | 275.8 | 192.6 | 83 | +226 | | | | 92 | 1,169 | +275 | 1,444 |
| 8 | A. S. no. 3 | 28′ 48″ | | | | | | | | | 48.3 | 43.38 | 7.50 | | | | |
| 9 | G. S. no. 5 | 34′ 30″ | 2.19 | 7.73 | 1.07 | 252.0 | 269.4 | 198.4 | 71 | +49 | | | | 83 | 1,205 | +59 | 1,264 |
| 10 | A. S. no. 4 | 34′ 40″ | | | | | | | | | 50.2 | 44.59 | 7.51 | | | | |
| 11 | Room Air | 35′ 40″ | | | | | | | | | c 51.0 | | | | | | |
| 12 | G. S. no. 6 | 38′ 18″ | 2.03 | 18.49 | 0.77 | 319.3 | 246.6 | 251.2 | −5 | +298 | | | | 56 | 1,526 | +363 | 1,889 |
| 13 | A. S. no. 5 | 38′ 38″ | | | | | | | | | 56.9 | 44.29 | 7.43 | | | | |
| 14 | G. S. no. 7 | 43′ 56″ | 1.82 | 18.52 | 0.69 | 321 | 220.6 | 252.6 | −32 | −129 | | | | 58 | 1,533 | −157 | 1,376 |
| 15 | G. S. no. 8 | 49′ 2″ | 1.85 | 18.64 | 0.75 | 301.8 | 224.8 | 273.3 | −12 | −129 | | | | 57 | 1,441 | −157 | 1,284 |
| 16 | A. S. no. 6 | 49′ 19″ | | | | | | | | | 47.7 | 44.53 | 7.44 | | | | |
| 17 | 850 cc. Saline | 50′ 17″ | | | | | | | | | c 47.0 | | | 57 | | | |

| | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 18 | A. S. no. 6A | 53′ 15″ | | | | | | | | +117 | | | 7.38 | | | | |
| 19 | G. S. no. 9 | 58′ 44″ | 2.34 | 18.30 | 0.84 | 339.4 | 285.4 | 267 | 18 | | | | | 102 | 1,622 | +142 | 1,764 |
| 20 | A. S. no. 7 | 58′ 44″ | | | | | | | | | 53.6 | 41.76 | 7.40 | | | | |
| 21 | G. S. no. 10 | 70′ 20″ | 2.18 | 18.43 | 0.82 | 323.8 | 265.5 | 254.9 | 11 | −99 | | | | 103 | 1,546 | +121 | 1,425 |
| 22 | A. S. no. 8 | 70′ 40″ | | | | | | | | | 45.7 | 42.75 | 7.42 | | | | |
| 23 | 9.774% $O_2$, 0.02% $CO_2$ | 74′ 56″ | | | | | | | | | c 42.7 | | | 100 | | | |
| 24 | G. S. 11 | 79′ 33″ | 2.28 | 7.69 | 1.11 | 256.8 | 285.2 | 202 | 83 | +178 | | | | 120 | 1,226 | +217 | 1,443 |
| 25 | G. S. no. 12 | 81′ 8″ | 2.33 | 7.55 | 1.04 | 278.6 | 290.5 | 219.2 | 71 | +178 | | | | 120 | 1,332 | +217 | 1,549 |
| 26 | A. S. no. 9 | 81′ 12″ | | | | | | | | | 50.2 | 41.69 | 7.49 | | | | |
| 27 | G. S. no. 13 | 85′ 15″ | 2.37 | 7.48 | 1.03 | 287.7 | 295.5 | 226.2 | 69 | +204 | | | | 116 | 1,374 | +248 | 1,622 |
| 28 | A. S. no. 10 | 85′ 15″ | | | | | | | | | 55.7 | 41.91 | 7.46 | | | | |
| 29 | Room Air | 86′ 38″ | | | | | | | | | c 57.0 | | | | | | |
| 30 | G. S. no. 14 | 88′ 25″ | 2.64 | 17.51 | 0.71 | 446.6 | 318.2 | 351.2 | −33 | 92 | | | | 80 | 2,132 | −73 | 2,059 |
| 31 | A. S. no. 10A | 88′ 25″ | | | | | | | | | | | 7.38 | | | | |
| 32 | G. S. no. 15 | 92′ 38″ | 2.21 | 17.92 | 0.67 | 397.0 | 265.5 | 312.2 | −47 | 92 | | | | 75 | 1,896 | −73 | 1,823 |
| 33 | A. S. no. 11 | 92′ 38″ | | | | | | | | | 53.3 | 41.40 | 7.39 | | | | |
| 34 | G. S. no. 16 | 101′ 11″ | 2.11 | 18.39 | 0.77 | 329.4 | 254.4 | 259.2 | −5 | 24 | | | | 76 | 1,574 | −30 | 1,544 |
| 35 | A. S. no. 12 | 101′ 11″ | | | | | | | | | 51.9 | 40.83 | 7.40 | | | | |

At 50 minutes 17 seconds—850 cc. of saline were injected with a decided improvement in the mean blood pressure. The augmented volume flow of blood was accompanied by increased gaseous exchange. Carbon dioxide elimination increased out of proportion to the increase in oxygen absorption and the expiratory quotient rose from 0.75 to 0.84 and 0.82 at gas samples 9 and 10. After a temporary rise in blood lactic acid a disappearance occurred.

At 74 minutes 56 seconds—9.77 per cent oxygen was administered again. As judged by the curves of oxygen consumption and carbon dioxide elimination the effects were in general the same, yet there is a difference in the relatively small shortage of oxygen absorption during anoxemia and the greater excess absorption during recovery. It might be concluded that increased volume flow of blood from the previous injection of saline solution wards off the dangers of oxygen shortage and keeps the oxidative mechanism more intact through better supply of oxygen and better removal of metabolites thereby making compensatory recovery more perfect. This agrees with our earlier results on hemorrhage and shock (Gesell, 1919).

On the whole this experiment showed a tendency towards parallelism of the curves of blood carbon dioxide capacity and blood lactic acid content. The pH changes of the blood were in general similar to those described in experiment 1—a decreased hydrogen ion concentration during anoxemia and a rise with a tendency towards recovery with each readministration of room air and with injection of saline solution. Comparing the initial and final hydrogen ion concentration of the blood, we find an increased acidity of 0.08 pH associated with an increase in blood lactic acid from 46 to 51.9 mgm. and a decrease in carbon dioxide capacity from 47.72 cc. to 40.83 cc.

Barring one short period of increasing lactic acid occurring with the first readministration of room air, which may possibly be due to an error in analysis, there are three pairs of changes in lactic acid—an increase and decrease in lactic acid with each period of anoxemia and recovery and an increase and decrease with the injection of saline solution. These changes are plotted as in the first experiment as $CO_2 \approx$ of lactic acid. The curve of non-oxidative carbon dioxide shows a large liberation in excess of that called for by oxidation during both periods of anoxemia. In the first period from the administration of low oxygen to gas sample 6 the carbon dioxide index was 0.60 and during the second period of anoxemia from the administration of low oxygen to readministration of room air the carbon dioxide index was 0.39. During the period of recovery between gas samples 6 and 8 carbon dioxide was retained. The ratio of carbon dioxide retained over the carbon dioxide equivalent of lactic acid decrease was 0.18.

The relation between non-oxidative carbon dioxide and the carbon dioxide equivalent of lactic acid with increase and decrease in blood lactic acid produced by intravenous injection of saline solution was quite different. While the carbon dioxide equivalent of the increased lactic acid is large, the increase in non-oxidative carbon dioxide eliminated is small. For this period of increased blood lactic acid the carbon dioxide index was only 0.19. Such findings suggest that the preliminary increase in blood lactic acid following saline injection is not due to a new liberation of acid from the precursor but rather to a freer washing out of the tissues where the acid has already accumulated. If this is true it is of course apparent that many of our deductions based on lactic acid changes as computed by our method are affected by changes in volume flow of blood. The increased elimination of carbon dioxide with its consequent increase in the expiratory quotient may thus possibly be accounted for by a freer flow of blood through the lungs and tissues, improving the transport of carbon dioxide, for in all probability the total lactic acid content of the body is diminished by increased volume flow of blood. There is, however, one more complication. Although we have assumed that the true respiratory quotient remains constant throughout an experiment this is done only for purposes of analysis. (By true respiratory quotient is meant the metabolic respiratory quotient and not the respiratory quotient as determined by the gaseous exchange at the lungs which is influenced by changes in pulmonary ventilation, volume flow of blood and changes in acid base equilibrium.) A change in the true respiratory quotient from intravenous injection must be considered as a possibility. It will be noted that during the period where lactic acid diminishes an excess of carbon dioxide above that called for by the initial expiratory quotient was still eliminated. Were it not for the freer elimination of carbon dioxide, which increased volume flow of blood effects, this might suggest an increased respiratory quotient.

Inspection of the curves of energy exchange shows that the results are somewhat different from those of experiment 1. In experiment 1 the energy exchange, aerobic plus anaerobic (lactic acid shift) fell considerably below the normal basal level during the periods of anoxemia. However, in experiment 2 there was very little reduction in the total energy dissipated during the first period of anoxemia and during the second period the total energy dissipated was actually increased above the preceding basal period. Since recovery was accompanied by excess energy dissipation, the total gross energy was greater than normal for both sets of observations. The relatively high dissipation of energy during anoxemia finds explanation in the relatively low oxygen lactic acid ratio and in the increased respiratory movements which call for greater oxidations. In the first administration of low oxygen where the energy exchange is the lower the ratio is 111. Where the energy exchange is higher the ratio is 64. The last administra-

tion more than the first indicates that the true basal energy expenditure (ignoring increased respiratory movements) may be increased by temporary anoxemia. Going next to the removal of lactic acid it will be seen that after the first period of anoxemia where the liberation of lactic acid per unit of oxygen shortage was least effective the non-oxidative removal was the more effective, and following the period of anoxemia where the liberation of lactic acid was greater the removal of lactic acid was relatively slow.

*Experiment 3.* In this experiment certain computations and comparisons are subject to greater error than in experiment 2 due to the steep gradient of the curves of oxygen consumption and carbon dioxide elimination. If the oxygen gradient prior to administration of low oxygen is extended through the period of recovery there is excessive oxygen absorption up to the second administration of low oxygen. If, on the other hand, a horizontal gradient is established from the point of the first administration of low oxygen we must assume a shortage of oxygen absorption from gas sample 7. While to be sure there is an absolute shortage, for the oxygen consumption is less than before the administration of low oxygen, a shortage does not exist if we picture the effects of administraton of low oxygen and readministration of room air as superimposed upon a metabolic gradient. But since it is probable that the curve of oxygen consumption would become more horizontal as the experiment proceeds it is impossible to make more than approximate deductions.

During the first period of anoxemia (9.81 per cent oxygen) oxygen absorption dropped off abruptly. The elimination of carbon dioxide continued virtually unchanged due to the marked increase in elimination of non-oxidative carbon dioxide. The source of this non-oxidative carbon dioxide is seen in the large increase in the blood lactic acid. During the rapid accumulation of lactic acid the non-oxidative carbon dioxide amounted to 155 cc. per kgm. hour while the carbon dioxide equivalent of lactic acid amounted to 533 cc. or three times as much. In terms of carbon dioxide (at gas sample 2) there was a potential formation of carbon dioxide during the initial period of 186 cc. (oxidative) + 533 cc. (non-oxidative) = 719 cc. or 2.21 times as much as during the administration of room air. Unless the carbon dioxide is carried off at a much more rapid rate than normal the tissues most probably turn acid. Interestingly enough it will be noted that the blood had turned more alkaline. On readministration of room air oxygen absorption and carbon dioxide elimination increased. Despite the relatively large increase in carbon dioxide elimination, the expiratory quotient suddenly decreased from the abnormally high value of 1.28 during anoxemia to the abnormally low value of 0.66 during oxidative recovery. This is explained again by non-oxidative removal of acid.

At 85 minutes 4 seconds a lower mixture of oxygen was administered

(8.00 per cent) for a period of 6 minutes 51 seconds. There was a greater reduction in oxidation, a relatively greater accumulation of lactic acid accompanied by an actual increased elimination of carbon dioxide. The increase in the expiratory quotient (2.84 − 0.76 = 2.08) was greater than that occurring on the administration of the higher oxygen mixture (1.35 − 0.75 = 0.60). This agrees with the results of experiment 1 and others that the greater the anoxemia the greater the increase in the expiratory quotient. This relation is shown in figure 8. On readministration of room air there was very poor recovery. Oxidations were only slightly

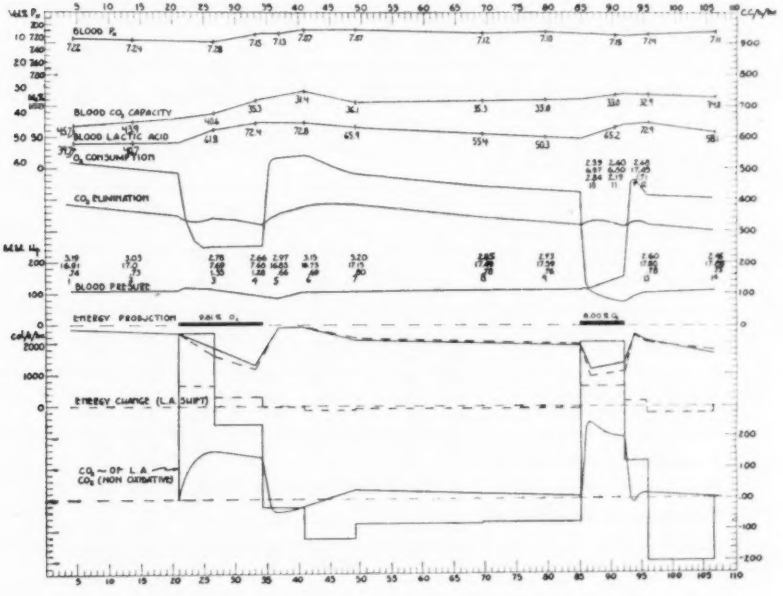



Fig. 5. Experiment 3

increased, still lactic acid disappeared at a rapid rate and the expiratory quotient diminished as before but not as markedly.

During the first perod of low oxygen (9.81 per cent) the elimination of non-oxidative carbon dioxide equaled 36 per cent of the carbon dioxide equivalent of lactic acid. Between the first and second administration of low oxygen the computed carbon dioxide index is 2.71. This exceedingly high value is difficult to explain unless we asume that the true respiratory quotient was increased by anoxemia as is indicated by gas samples 7, 8 and 9 and that a relatively large amount of the lactic acid which dis-

TABLE 3

*Experiment 3*

| OBSERVATION | PROCEDURE | TIME | PER CENT OF EXPIRED $CO_2$ | PER CENT OF EXPIRED $O_2$ | EXPIRATORY QUOTIENT | $O_2$ CONSUMPTION IN CC. PER KGM. HR. | $CO_2$ ELIMINATION IN CC. PER KGM. HR. | $CO_2$ ELIMINATION FROM OXIDATIONS BASED ON E.Q. IN CC. PER KGM. HR. | DIFFERENCE BETWEEN ACTUAL AND THEORETICAL $CO_2$ ELIMINATION | $CO_2$ EQUIVALENT OF CHANGE OF LACTIC ACID | LACTIC ACID IN MGM. PER 100 CC. ARTERIAL BLOOD | $CO_2$ CAPACITY OF ARTERIAL BLOOD IN VOL. PER CENT | pH OF ARTERIAL BLOOD | MEAN BLOOD PRESSURE | OXIDATIVE ENERGY IN SMALL CALORIES PER KGM. HR. | NON-OXIDATIVE ENERGY IN SMALL CALORIES PER KGM. HR. | OXIDATIVE + NON-OXIDATIVE ENERGY IN SMALL CALORIES PER KGM. HR. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 |
| 1 | G. S. no. 1 | 3′ 54″ | 3.19 | 16.91 | 0.74 | 519.6 | 384.2 | | | | | | | 110 | 2,452 | | |
| 2 | A. S. no. 1 | 4′ 27″ | | | | | | | | | 39.7 | 45.74 | 7.22 | | | | |
| 3 | G. S. no. 2 | 13′ 31″ | 3.03 | 17.07 | 0.73 | 500.1 | 364.7 | | | | | | | 110 | 2,363 | | |
| 4 | A. S. no. 2 | 13′ 48″ | | | | | | | | | 40.7 | 43.88 | 7.24 | | | | |
| 5 | 9.81% $O_2$, 0.01% $CO_2$ | 20′ 58″ | | | | | | | | | c 41.7 | | | 110 | | | |
| 6 | G. S. no. 3 | 26′ 40″ | 2.78 | 7.69 | 1.35 | 252.0 | 340.7 | 186 | 155 | +533 | | | | 114 | 1,192 | 648 | 1,840 |
| 7 | A. S. no. 3 | 26′ 37″ | | | | | | | | | 61.8 | 40.62 | 7.28 | | | | |
| 8 | G. S. no. 4 | 33′ 9″ | 2.66 | 7.68 | 1.28 | 254.0 | 325.4 | 187.6 | 138 | +239 | | | | 95 | 1,200 | 291 | 1,491 |
| 9 | A. S. no. 4 | 33′ 15″ | | | | | | | | | 72.4 | 35.32 | 7.15 | | | | |
| 9a | Room Air | 34′ 17″ | | | | | | | | | c 74.0 | | | | | | |
| 10 | G. S. no. 5 | 36′ 50″ | 2.97 | 16.83 | 0.66 | 535.0 | 354.6 | 395.5 | −41 | −27 | | | | 84 | 2,530 | −33 | 2,497 |
| 11 | A. S. no. 4A pH | 36′ 57″ | | | | | | | | | | | 7.13 | | | | |
| 12 | G. S. no. 6 | 40′ 48″ | 3.15 | 16.75 | 0.69 | 542.9 | 376.2 | 401.2 | −25 | −27 | | | | 105 | 2,568 | −33 | 2,535 |
| 13 | A. S. no. 5 | 40′ 53″ | | | | | | | | | 72.8 | 31.40 | 7.07 | | | | |
| 14 | G. S. no. 7 | 49′ 6″ | 3.20 | 17.15 | 0.80 | 483 | 385.1 | 357 | +28 | −126 | | | | 107 | 2,281 | −153 | 2,128 |
| 15 | A. S. no. 6 | 49′ 6″ | | | | | | | | | 65.9 | 36.08 | 7.07 | | | | |
| 16 | Stopped drum as canula col. clogged | 50′ 20″ | | | | | | | | | | | | | | | |

| | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 17 | Start drum | 65′ | | | | | | | | | | | | | | | |
| 18 | G. S. no. 8 | 69′ 23″ | 2.85 | 17.49 | 0.78 | 441.1 | 342.7 | 326 | +17 | −78 | | | | 110 | 2,084 | −95 | 1,989 |
| 19 | A. S. no. 7 | 69′ 22″ | | | | | | | | | 55.4 | 35.28 | 7.12 | | | | |
| 20 | G. S. no. 9 | 79′ 10″ | 2.73 | 17.59 | 0.76 | 429.4 | 327.7 | 317.2 | 10 | −76 | | | | 110 | 2,030 | −93 | 1,937 |
| 21 | A. S. no. 8 | 79′ 23″ | | | | | | | | | 50.3 | 34.99 | 7.10 | | | | |
| 22 | 8.00% $O_2$, 0.00% $CO_2$ | 85′ 4″ | | | | | | | | | c 47.3 | | | 110 | | | |
| 23 | G. S. no. 10 | 86′ 40″ | 2.59 | 6.97 | 2.84 | 115.4 | 328.2 | 84.2 | 244 | 502 | | | | 104 | 545 | +610 | 1,153 |
| 24 | G. S. no. 11 | 90′ 18″ | 2.40 | 6.80 | 2.19 | 140.8 | 308.3 | 104 | 204 | 502 | | | | 76 | 665 | +610 | 1,275 |
| 25 | A. S. no. 9 | 90′ 25″ | | | | | | | | | 65.2 | 33.02 | 7.15 | | | | |
| 26 | Room Air | 91′ 55″ | | | | | | | | | c 70.0 | | | | | | |
| 27 | G. S. no. 12 | 93′ 33″ | 2.68 | 17.45 | 0.71 | 461.2 | 327.7 | 341 | −14 | 112 | | | | 87 | 2,180 | +136 | 2,316 |
| 28 | G. S. no. 13 | 95′ 39″ | 2.60 | 17.80 | 0.78 | 411.0 | 318.9 | 303.9 | +15 | 112 | | | | 100 | 1,943 | +136 | 2,079 |
| 29 | A. S. no. 10 | 95′ 47″ | | | | | | | | | 72.9 | 32.89 | 7.14 | | | | |
| 30 | G. S. no. 14 | 106′ 16″ | 2.45 | 17.88 | 0.75 | 402.0 | 299.3 | 297 | +2 | −205 | | | | 107 | 1,900 | −249 | 1,651 |
| 31 | A. S. no. 11 | 106′ 33″ | | | | | | | | | 58.1 | 33.96 | 7.11 | | | | |
| 32 | G. S. no. 15 | 129′ 0″ | 2.41 | 17.98 | 0.76 | 389.2 | 295.2 | 287.8 | +7 | +5 | | | | 105 | 1,840 | +6 | 1,846 |
| 33 | A. S. no. 12 | 129′ 0″ | | | | | | | | | 58.8 | 35.07 | 7.08 | | | | |

appeared during recovery was removed by oxidative processes. During the second administration of low oxygen while the formation of lactic acid is rapid the carbon dioxide index again approached more usual values − 0.45. For the more inclusive period between the administration of low oxygen and gas sample 14 the value increased again to 1.15. It might be well to consider the probability of formation of fixed acids other than lactic acid.

The oxygen lactic acid ratios for this experiment have been computed in two ways. In one, the gradient of oxygen consumption has been ignored and as base for comparison a horizontal line was extended from the point at which low oxygen was administered. In the other, the initial gradient was extended. Ignoring the gradient, the ratio of oxygen shortage to lactic acid increase during the first period of anoxemia was 144 indicating a fairly rapid formation of lactic acid. This method, however, gives a very short period of excess absorption of oxygen and after gas sample 7 there is a definite shortage of oxygen accompanied by a rapid removal of lactic acid. Thus for the total period of anoxemia and recovery up to the second low oxygen the ratio is very high indicating exceedingly slow formation of lactic acid. In attempting a correction by extending the oxygen consumption gradient for the period of anoxemia the quotient is approximately the same, 135 as compared with 144. For the period of recovery from room air to the second administration of low oxygen the ratio of oxygen excess to lactic acid decrease is 170, and for the period of anoxemia plus the period of recovery up to gas sample 7 the ratio of oxygen shortage to lactic acid increase is 122. The results obtained with the gradient are in far better agreement with the results of the preceding experiments. The oxygen lactic acid ratios for the second low alveolar oxygen and recovery computed by the two methods are in better agreement for the gradient is less. For the period of anoxemia the ratio of oxygen shortage to lactic acid increase was 139 with the horizontal gradient and 138 with the initial gradient and for the total period of anoxemia and recovery the ratios were 325 and 285 respectively.

Experiment 4 shows effects of administration of low oxygen, of saline solution and of the simultaneous administration of carbon dioxide and sodium bicarbonate. For the present only the effects of lowered alveolar oxygen will be considered.

During the first fifteen minutes of the experiment preceding the administration of low oxygen, the mean blood pressure fell steadily and oxygen consumption and carbon dioxide elimination diminished, the expiratory quotient, however, remained virtually unchanged at 0.83. Strangely, too, there was no change in the blood picture indicating the usual effects of anoxemia. But with the administration of low oxygen, oxygen absorption was markedly reduced. There was only a small reduction in

expired carbon dioxide and the expiratory quotient rose from 0.83 to 2.13. Blood lactic acid increased rapidly accompanied by a corresponding decrease in blood carbon dioxide capacity and a marked decrease in the hydrogen ion concentration of the blood.

Elimination of non-oxidative carbon dioxide increased decidedly. During the period of anoxemia the carbon dioxide index was 0.50. Readministration of room air led to excessive oxygen absorption, excess elimination of carbon dioxide, a retention of carbon dioxide, a reduction of the expiratory quotient below the initial level, a reduction in the blood lactic acid and an increase in the blood carbon dioxide capacity.

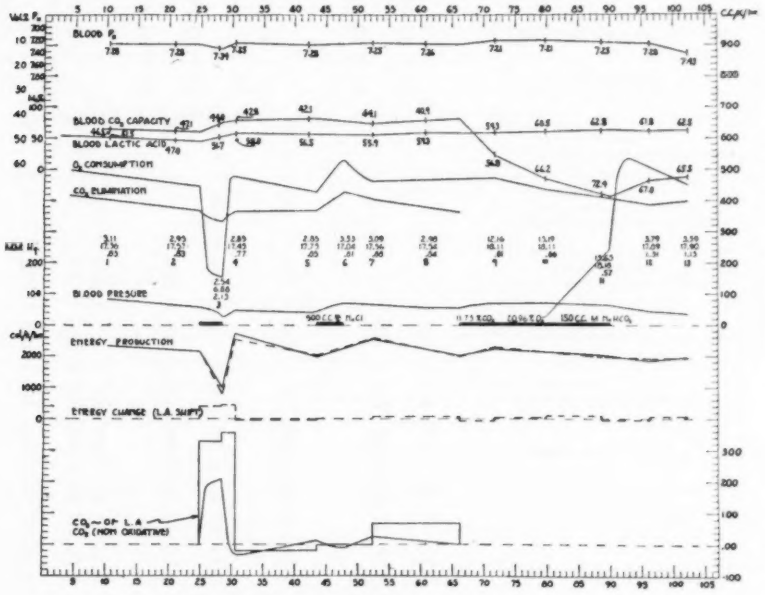



Fig. 6. Experiment 4

Experiment 5 shows effects of two administrations of an 8.47 per cent oxygen mixture. While in general the results resemble those of other experiments there are some differences worthy of note. On the first administration of low oxygen, oxygen absorption fell to an exceptionally low value. Carbon dioxide elimination remained approximately constant; the expiratory quotient rose from 0.87 to 4.34. Blood lactic acid increased and also the hydrogen ion concentration of the blood. The increase in carbon dioxide capacity of the blood is a striking and unexpected phenomenon. On readministration of room air both oxygen absorption

TABLE 4

*Experiment 4*

| OBSERVATION | PROCEDURE | TIME | PER CENT OF EXPIRED $CO_2$ | PER CENT OF EXPIRED $O_2$ | EXPIRATORY QUOTIENT | $O_2$ CONSUMPTION IN CC. PER KGM. HR. | $CO_2$ ELIMINATION IN CC. PER KGM. HR. | $CO_2$ ELIMINATION FROM OXIDATIONS BASED ON E.Q. IN CC. PER KGM. HR. | DIFFERENCE BETWEEN ACTUAL AND THEORETICAL $CO_2$ ELIMINATION | $CO_2$ EQUIVALENT OF CHANGE OF LACTIC ACID | LACTIC ACID IN MGM. PER 100 CC. ARTERIAL BLOOD | $CO_2$ CAPACITY OF ARTERIAL BLOOD IN VOL. PER CENT | pH OF ARTERIAL BLOOD | MEAN BLOOD PRESSURE | OXIDATIVE ENERGY IN SMALL CALORIES PER KGM. HR. | NON-OXIDATIVE ENERGY IN SMALL CALORIES PER KGM. HR. | OXIDATIVE + NON-OXIDATIVE ENERGY IN SMALL CALORIES PER KGM. HR. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 |
| | 450 cc. Saline | | | | | | | | | | | | | | | | |
| 1 | G. S. no. 1 | 10′ 13″ | 3.11 | 17.36 | 0.828 | 483.6 | 400.2 | | | | | | | 84 | 2,338 | | |
| 2 | A. S. no. 1 | 10′ 25″ | | | | | | | | | 51.5 | 46.48 | 7.28 | | | | |
| 3 | G. S. no. 2 | 20′ 47″ | 2.93 | 17.57 | 0.826 | 456.4 | 376.9 | | | | | | | 66 | 2,208 | | |
| 4 | A. S. no. 2 | 20′ 54″ | | | | | | | | | 47.0 | 47.10 | 7.28 | | | | |
| 5 | 0.01% $CO_2$, 8.175% $O_2$ | 24′ 50″ | | | | | | | | | c 45.1 | | | 58 | | | |
| 6 | G. S. no. 3 | 27′ 53″ | 2.54 | 6.88 | 2.135 | 157.3 | 336 | 131 | 205 | +329 | | | | | 755 | +399 | 1,154 |
| 7 | A. S. no. 3 | 27′ 51″ | | | | | | | | | 51.7 | 44.03 | 7.34 | | | | |
| 8 | Room Air | 28′ 25″ | | | | | | | | | c 53 | | | 28 | | | |
| 9 | G. S. no. 4 | 30′ 29″ | 2.85 | 17.45 | 0.766 | 476.5 | 364.9 | 394.9 | −30 | +356 | | | | 48 | 2,302 | +423 | 2,725 |
| 10 | A. S. no. 4 | 30′ 35″ | | | | | | | | | 58.0 | 42.76 | 7.25 | | | | |
| 11 | G. S. no. 5 | 42′ 6″ | 2.85 | 17.73 | 0.846 | 432.6 | 366.1 | 358 | +8 | −19 | | | | 42 | 2,090 | −24 | 2,066 |
| 12 | A. S. no. 5 | 42′ 6″ | | | | | | | | | 56.5 | 42.06 | 7.28 | | | | |
| 13 | 500 cc. Saline | 43′ 29″ | | | | | | | | | | | | 43 | | | |
| 14 | G. S. no. 6 | 47′ 53″ | 3.33 | 17.04 | 0.810 | 527.6 | 427.5 | 436.5 | −9 | 0 | | | | 70 | 2,550 | | 2,550 |
| 15 | G. S. no. 7 | 52′ 22″ | 3.09 | 17.56 | 0.879 | 461.9 | 406 | 382 | +24 | 0 | | | | 65 | 2,231 | | 2,231 |
| 16 | A. S. no. 6 | 52′ 22″ | | | | | | | | | 55.9 | 44.08 | 7.25 | | | | |
| 17 | G. S. no. 8 | 60′ 52″ | 2.98 | 17.54 | 0.835 | 467.8 | 390.8 | 382.2 | +9 | 62 | | | | 56 | 2,261 | +73 | 2,334 |
| 18 | A. S. no. 7 | 60′ 49″ | | | | | | | | | 59.3 | 40.86 | 7.26 | | | | |

and carbon dioxide elimination were again increased. The reduction in the expiratory quotient was relatively small corresponding perhaps with the slow rate of disappearance of blood lactic acid. Comparing the non-oxidative carbon dioxide with the carbon dioxide equivalent of lactic acid it will be seen that the relative area of the non-oxidative carbon dioxide curve is excessively large—for the first time larger than the area of the curve of carbon dioxide equivalent of lactic acid during anoxemia. From low oxygen to gas sample 4 the carbon dioxide index was 1.07, from gas sample 4 to room air it was 1.20 and for the total period from low oxygen

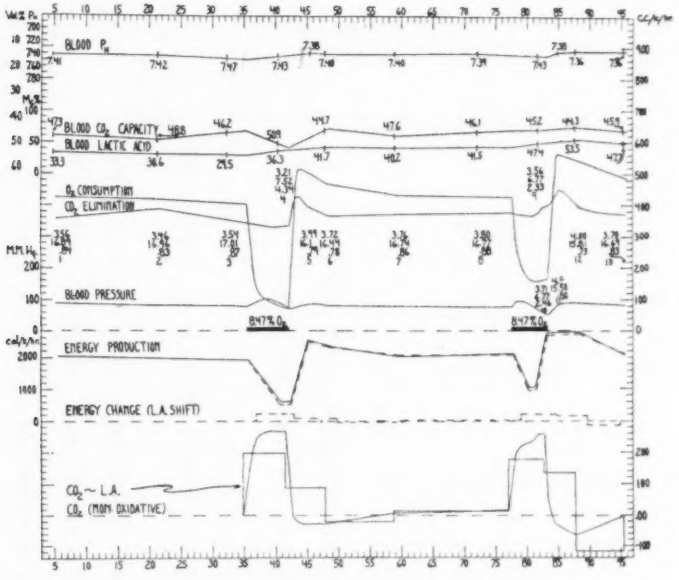



Fig. 7. Experiment 5

to room air it was 1.09. For the total period from low oxygen to gas sample 8 which includes recovery, the coefficient was 0.80. This is still a very high figure for in general especially for the longer and more inclusive periods the carbon dioxide index was approximately 0.45. It is, therefore, of interest to know what mechanisms have been at work to raise the carbon dioxide index so excessively. An exceptionally large increase in volume flow of blood might possibly contribute. The records show an increase in carotid flow. It is also possible that in this particular animal lactic acid migrated from the tissues to the blood slowly compared with the rapid diffusion of carbon dioxide. That is, a relatively large amount of lactic

TABLE 5

*Experiment 5*

| OBSERVATION | PROCEDURE | TIME | PER CENT OF EXPIRED $CO_2$ | PER CENT OF EXPIRED $O_2$ | EXPIRATORY QUOTIENT | $O_2$ CONSUMPTION IN CC. PER KGM. HR. | $CO_2$ ELIMINATION IN CC. PER KGM. HR. | $CO_2$ ELIMINATION FROM OXIDATIONS BASED ON E.Q. IN CC. PER KGM. HR. | DIFFERENCE BETWEEN ACTUAL AND THEORETICAL $CO_2$ ELIMINATION | $CO_2$ EQUIVALENT OF CHANGE OF LACTIC ACID | LACTIC ACID IN MGM. PER 100 CC. ARTERIAL BLOOD | $CO_2$ CAPACITY OF ARTERIAL BLOOD IN VOL. PER CENT | pH OF ARTERIAL BLOOD | MEAN BLOOD PRESSURE | OXIDATIVE ENERGY IN SMALL CALORIES PER KGM. HR. | NON-OXIDATIVE ENERGY IN SMALL CALORIES PER KGM. HR. | OXIDATIVE + NON-OXIDATIVE ENERGY IN SMALL CALORIES PER KGM. HR. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 |
| 1 | G. S. no. 1 | 4′ 42″ | 3.56 | 16.89 | 0.84 | 424.0 | 356.2 | 356 | | | | | | 88 | 2,056 | | |
| 2 | A. S. no. 1 | 4′ 55″ | | | | | | | | | 33.3 | 47.31 | 7.41 | | | | |
| 3 | G. S. no. 2 | 21′ 1″ | 3.46 | 16.96 | 0.83 | 417.8 | 346.0 | 351 | | | | | | 80 | 2,026 | −30 | 1,996 |
| 4 | A. S. no. 2 | 21′ 21″ | | | | | | | | | 30.6 | 48.83 | 7.42 | | | | |
| 5 | G. S. no. 3 | 32′ 6″ | 3.54 | 17.01 | 0.87 | 404.0 | 350 | 339.2 | | | | | | 80 | 1,959 | −22 | 1,937 |
| 6 | A. S. no. 3 | 32′ 19″ | | | | | | | | | 29.5 | 46.18 | 7.47 | | | | |
| 7 | 8.47% $O_2$, 0.00% $CO_2$ | 34′ 55″ | | | | | | | | | c 29.2 | | | 77 | | | |
| 8 | G. S. no. 4 | 40′ 17″ | 3.21 | 7.52 | 4.34 | 76.29 | 330.9 | 64 | 267 | +197 | | | | 85 | 370 | +240 | 610 |
| 9 | A. S. no. 4 | 40′ 19″ | | | | | | | | | 36.3 | 50.94 | 7.43 | | | | |
| 10 | Room Air | 41′ 33″ | | | | | | | | | c 38 | | | 74 | | | |
| 11 | G. S. no. 5 | 44′ 24″ | 3.99 | 16.10 | 0.79 | 504.6 | 396.2 | 424 | −28 | +88 | | | | 82 | 2,448 | +107 | 2,555 |
| 12 | A. S. no. 4A$^{pH}$ | 45′ 31″ | | | | | | | | | | | 7.38 | | | | |
| 13 | G. S. no. 6 | 47′ 52″ | 3.72 | 16.44 | 0.78 | 465.8 | 364.1 | 391 | −27 | +88 | | | | 78 | 2,260 | +107 | 2,367 |
| 14 | A. S. no. 5 | 47′ 52″ | | | | | | | | | 41.7 | 44.73 | 7.40 | | | | |
| 15 | G. S. no. 7 | 58′ 57″ | 3.76 | 16.74 | 0.86 | 429.6 | 369.1 | 361 | +8 | −21 | | | | 76 | 2,082 | −25 | 2,057 |
| 16 | A. S. no. 6 | 58′ 57″ | | | | | | | | | 40.2 | 47.58 | 7.40 | | | | |
| 17 | G. S. no. 8 | 72′ 3″ | 3.80 | 16.77 | 0.88 | 425.0 | 373.4 | 357 | +16 | +15 | | | | 74 | 2,061 | +18 | 2,079 |

| | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 18 | A. S. no. 7 | 72′ 3″ | | | | | | | | | 41.5 | 46.13 | 7.39 | | | | |
| 19 | 8.47% $O_2$, 0.00% $CO_2$ | 77′ 3″ | | | | | | | | | c 42.0 | | | 75 | | | |
| 20 | G. S. no. 9 | 80′ 27″ | 3.56 | 6.77 | 2.33 | 155.9 | 362.7 | 131 | 232 | +176 | | | | 80 | 756 | +213 | 969 |
| 21 | G. S. no. 10 | 81′ 40″ | 3.71 | 6.77 | 2.46 | 157.7 | 387.5 | 132.5 | 255 | +176 | | | | 60 | 767 | +213 | 980 |
| 22 | A. S. no. 8 | 81′ 40″ | | | | | | | | | 47.4 | 45.23 | 7.43 | | | | |
| 23 | Room Air | 82′ 41″ | | | | | | | | | c 49 | | | 50 | | | |
| 24 | G. S. no. 11 | 84′ 25″ | 4.57 | 15.50 | 0.80 | 560.5 | 447.0 | 471 | −24 | +135 | | | | 82 | 2,720 | +164 | 2,884 |
| 25 | A. S. no. 8A$^{pH}$ | 84′ 17″ | | | | | | | | | | | 7.38 | | | | |
| 26 | G. S. no. 12 | 87′ 36″ | 4.00 | 15.81 | 0.73 | 540.4 | 393.4 | 454 | −61 | +135 | | | | 87 | 2,621 | +164 | 2,785 |
| 27 | A. S. no. 9 | 87′ 41″ | | | | | | | | | 53.5 | 44.28 | 7.36 | | | | |
| 28 | G. S. no. 13 | 95′ 8″ | 3.70 | 16.69 | 0.83 | 441.2 | 366.2 | 370.5 | −4 | −117 | | | | 80 | 2,138 | −143 | 1,995 |
| 29 | A. S. no. 10 | 95′ 25″ | | | | | | | | | 47.7 | 45.86 | 7.36 | | | | |

TABLE 6

*Summary of carbon dioxide indices and oxygen-lactic acid ratios*

| PERIOD | VARIATIONS IN $O_2$ CONSUMPTION, CMM./KGM. (+ = EXCESS, − = SHORTAGE) | VARIATIONS IN LACTIC ACID, MGM./KGM. (+ = INCREASE, − = DECREASE) | $O_2$ SHORTAGE, CMM./KGM. / LACTIC ACID INCREASE, MGM./KGM. | $O_2$ EXCESS, CMM./KGM. / LACTIC ACID DECREASE, MGM./KGM. | NON-OXIDATIVE $CO_2$, CC./KGM. (+ = ELIMINATION, − = RETENTION) | $CO_2$ ~ LACTIC ACID CHANGES, CC./KGM. (+ = INCREASING LACTIC ACID, − = DECREASING LACTIC ACID) | + NON-OXIDATIVE $CO_2$ / $CO_2$ ~ LACTIC ACID INCREASE | $CO_2$ RETENTION / $CO_2$ ~ LACTIC ACID DECREASE |
|---|---|---|---|---|---|---|---|---|
| **Experiment 1** | | | | | | | | |
| Low $O_2$ to G. S. 3 | −16,470 | +74 | 223 | | +11.24 | +18.5 | 0.61 | |
| G. S. 3 to G. S. 4 | −18,740 | +90 | 208 | | +12.52 | +22.5 | 0.55 | |
| G. S. 4 to Room Air | −4,750 | +20 | 238 | | +3.05 | +5.00 | 0.61 | |
| Room Air to G. S. 5 | +1,830 | −5 | | 366 | −1.55 | −1.25 | | 1.24 |
| G. S. 5 to G. S. 6 | +3,100 | −5 | | 620 | −1.76 | −1.25 | | 1.42 |
| G. S. 6 to G. S. 7 | +3,860 | +24 | | | −0.69 | +6.00 | | |
| G. S. 7 to Saline injection | −440 | +16 | 28 | | +0.04 | +4.00 | 0.01 | |
| Low $O_2$ to Room Air | −39,960 | +184 | 217 | | +26.81 | +46.00 | 0.58 | |
| Room Air to Saline injection | +8,170 | +30 | | | −3.96 | +7.50 | | |
| Low $O_2$ to Saline injection | −31,790 | +214 | 148 | | +22.85 | +53.50 | 0.43 | |
| Saline to 2nd Low $O_2$ | +16,130 | −97 | | 163 | +12.60 | −24.25 | | |
| 2nd Low $O_2$ to G. S. 11 | −12,370 | +145 | 85 | | +8.58 | +36.25 | 0.24 | |
| G. S. 11 to Room Air | −9,420 | +74 | 127 | | +6.15 | +18.50 | 0.33 | |
| 2nd Room Air to G. S. 12 | −5,590 | −254 | | | +4.88 | −5.50 | | |
| G. S. 12 to G. S. 13 | +3,850 | −32 | | 120 | +1.68 | −8.00 | | |
| G. S. 13 to G. S. 14 | +7,860 | −58 | | 135 | −3.11 | −14.50 | | 0.21 |
| 2nd Low $O_2$ to Room Air | −21,790 | +219 | 100 | | +14.73 | +54.75 | 0.27 | |
| 2nd Room Air to G. S. 14 | +6,120 | −112 | | 55 | +3.45 | −28.00 | | |
| 2nd Low $O_2$ to G. S. 14 | −15,760 | +107 | 146 | | +18.18 | +26.75 | 0.68 | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Experiment 2** | | | | | | | | |
| Low $O_2$ to Room Air | −13,320 | +120 | 111 | | +17.96 | +30.00 | 0.60 | |
| G. S. 6 to G. S. 8 | +2,740 | −80 | | 34 | −3.61 | −20.00 | | 0.18 |
| Room Air to G. S. 8 | +3,150 | −30 | | 105 | −2.60 | −7.50 | | 0.35 |
| Low $O_2$ to G. S. 8 | −10,170 | +90 | 113 | | +15.36 | +22.50 | 0.69 | |
| Saline to G. S. 9 | +6,920 | +60 | | | +2.92 | +15.00 | 0.19 | |
| G. S. 9 to G. S. 10 | +7,800 | −60 | | 130 | +2.83 | −15.00 | | |
| Saline to G. S. 10 | +14,720 | 0 | | | +5.75 | 0.00 | | |
| 2nd Low $O_2$ to 2nd Room Air | −9,660 | +150 | 64 | | +14.60 | +37.50 | 0.39 | |
| 2nd Room Air to G. S. 16 | +15,790 | −50 | 316 | | −6.78 | −12.50 | 0.54 | |
| 2nd Low $O_2$ to G. S. 16 | +6,180 | +100 | | | +7.88 | +25.00 | 0.31 | |
| **Experiment 3. Without gradient** | | | | | | | | |
| Low $O_2$ to Room Air | −47,420 | +330 | 144 | | +29.83 | +82.50 | 0.36 | |
| Room Air to 2nd Low $O_2$ | −15,900 | −268 | | | +11.36 | −67.00 | | |
| 1st Low $O_2$ to 2nd Low $O_2$ | −63,320 | +62 | 1021 | | +41.19 | +15.50 | 2.71 | |
| 2nd Low $O_2$ to 2nd Room Air | −31,820 | +228 | 139 | | +25.89 | +57.00 | 0.45 | |
| 2nd Room Air to G. S. 14 | −3,940 | −118 | | | +5.57 | −29.50 | | |
| 2nd Low $O_2$ to G. S. 14 | −35,760 | +110 | 325 | | +31.46 | +27.50 | 1.15 | |
| **Experiment 3. Using gradients** | | | | | | | | |
| Low $O_2$ to Room Air | +44,490 | +330 | 135 | | | | | |
| Room Air to 2nd Low $O_2$ | +45,430 | −268 | | 170 | | | | |
| 1st Low $O_2$ to G. S. 7 | −30,300 | +249 | 122 | | | | | |
| 2nd Low $O_2$ to 2nd Room Air | −31,360 | +228 | 138 | | | | | |
| 2nd Low $O_2$ to G. S. 14 | −31,380 | +110 | 285 | | | | | |
| **Experiment 4** | | | | | | | | |
| Low $O_2$ to Room Air | −13,930 | +80 | 174 | | +10.05 | +20.00 | 0.50 | |
| Low $O_2$ to G. S. 5 | −14,580 | +115 | 127 | | +8.97 | +28.75 | 0.31 | |
| Saline to G. S. 8 | +13,750 | +30 | | | +1.95 | +7.5 | 0.26 | |

TABLE 7

*Summary of carbon dioxide indices and oxygen-lactic acid ratios*

| PERIOD | VARIATIONS IN $O_2$ CONSUMPTION, CMM./KGM. (+ = EXCESS, − = SHORTAGE) | VARIATIONS IN LACTIC ACID, MGM./KGM. (+ = INCREASE, − = DECREASE) | $O_2$ SHORTAGE, CMM./KGM. / LACTIC ACID INCREASE, MGM./KGM. | $O_2$ EXCESS, CMM./KGM. / LACTIC ACID DECREASE, MGM./KGM. | NON-OXIDATIVE $CO_2$, CC./KGM. (+ = ELIMINATION, − = RETENTION) | $CO_2$ ~ LACTIC ACID CHANGES, CC./KGM. (+ = INCREASING LACTIC ACID, − = DECREASING LACTIC ACID) | + NON-OXIDATIVE $CO_2$ / $CO_2$ ~ LACTIC ACID INCREASE | $CO_2$ RETENTION / $CO_2$ ~ LACTIC ACID DECREASE |
|---|---|---|---|---|---|---|---|---|
| **Experiment 5** | | | | | | | | |
| 1st Low $O_2$ to Room Air | −29,910 | +90 | 333 | | +24.55 | +22.50 | 1.09 | |
| 1st Room Air to G. S. 8 | +20,110 | +35 | | | +1.00 | +8.75 | 0.11 | |
| 1st Low $O_2$ to G. S. 8 | −9,800 | +125 | 79 | | +25.55 | +31.25 | 0.80 | |
| 2nd Low $O_2$ to 2nd Room Air | −21,440 | +70 | 309 | | +19.65 | +17.50 | 1.12 | |
| 2nd Room Air to G. S. 13 | +14,000 | −10 | | 1400 | −1.46 | −2.50 | | 0.58 |
| 2nd Low $O_2$ to G. S. 13 | −4,640 | +60 | 77 | | +18.20 | +15.00 | 1.21 | |

acid may have been retained in the tissues and the carbon dioxide which was formed may have reached the blood and been eliminated in the lungs. There is some evidence suggesting this possibility, for example, the oxygen lactic acid ratio during the short period of anoxemia is very high, 333, indicating a very small formation of lactic acid during oxygen shortage. When, however, the oxygen lactic acid ratio is computed for the more inclusive period from low oxygen to gas sample 8 the ratio is 79 indicating a more copious formation of lactic acid. This smaller ratio may be due in part to a more complete diffusion of lactic acid into the blood stream.

Energy production fell off during anoxemia as is commonly the case but during the subsequent period of readministration of room air there was a prolonged period of hyper-oxidation which raised the sum total of energy dissipated during anoxemia and recovery above the normal.

All in all, the results of the second administration are like those of the first. Note that the second administration did not lower the rate of oxygen absorption as much as the first administration and that during anoxemia there was a tendency to check the impairment of oxidations. The smaller reduction in oxidations was accompanied by the smaller increase in the expiratory quotient. The carbon dioxide index was again very high for the period of anoxemia (1.12), but in this case was still higher for the total period from low oxygen to gas sample 13. The total energy production for the period of anoxemia and recovery was again greater than normal.

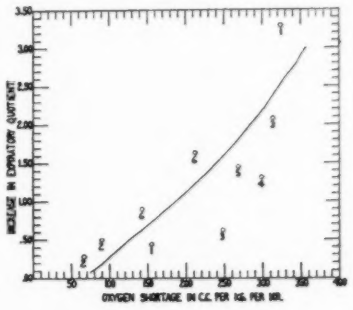



Fig. 8

## SUMMARY

This paper is the first of a series in which numerous factors relating to respiratory control are simultaneously studied.

Tidal air, pulmonary ventilation, respiratory movements, carbon dioxide and oxygen content of expired air, total elimination of carbon dioxide and total absorption of oxygen, expiratory quotient, lactic acid content of the blood, carbon dioxide capacity of the blood, hydrogen ion concentration of the blood, volume flow of blood, mean arterial blood pressure, oxidative energy, non-oxidative energy, the relation of shortage of oxygen consumption to accumulated lactic acid, the relation of excess oxygen consumption to the disappearance of lactic acid, the relation of total elimination of carbon dioxide to that required by oxidations, the relation of the lactic acid content of the body to the liberation and retention of carbon dioxide,

the proportion of fixed acid combining with bicarbonate and the proportion reacting with other buffer systems were studied.

The object of this series of experiments is a further evaluation of the various factors in respiratory control with particular reference to a differentiation in the effects of hydrogen ion concentration and the effects of impaired oxidations independent of changes in acid base equilibrium.

In the first of this series of experiments room air and gaseous mixtures low in oxygen were administered by constant artificial ventilation during pneumo-thorax.

With administration of gaseous mixtures low in oxygen the consumption of oxygen invariably decreased. With the readministration of room air, oxygen consumption increased to super normal levels and then returned to normal values. The excess oxygen consumption was, as a rule, considerably less than the preceding shortage.

With the same administrations the changes in carbon dioxide elimination were considerably smaller. While the absolute amount of carbon dioxide eliminated during lowered alveolar oxygen was commonly decreased, in some experiments it remained virtually unchanged and in others it increased above the normal level. With readministration of room air carbon dioxide elimination almost invariably increased above the normal level. In one experiment in which carbon dioxide elimination was above normal during lowered alveolar oxygen (expt. 2) the primary effect of readministration of room air was a reduction in carbon dioxide elimination.

These alterations in gaseous exchange resulted in an increased expiratory quotient during lowered alveolar oxygen and to a decrease below normal soon after readministration of room air. The greater the oxygen shortage the greater was the increase in the expiratory quotient.

Lowered alveolar oxygen was accompanied by an increase in blood lactic acid and a decrease in carbon dioxide capacity of the blood. Readministration of room air was associated with a decrease or a tendency towards a decrease of blood lactic acid and an increase or tendency towards an increase in blood carbon dioxide capacity.

Despite the fact that pulmonary ventilation was constant and that the carbon dioxide capacity decreased during lowered alveolar oxygen the hydrogen ion concentration of the arterial blood diminished. With readministration of room air the hydrogen ion concentration increased above normal and then tended to return to normal.

Lowered alveolar oxygen increased the carotid flow of blood and readministration of room air diminished the flow.

Since pulmonary ventilation was constant the changes in the expiratory quotient were attributed to changes in lactic acid metabolism and volume flow of blood. It is suggested that the increased expiratory quotient during anoxemia resulting from reaction of newly liberated lactic acid with

bicarbonate base is slightly augmented by the freer ventilation of the body resulting from increased flow of blood. Similarly the decreased volume flow of blood with readministration of room air should augment the retention of carbon dioxide produced by the shift of lactic acid back to the precursor state.

It is suggested that the true respiratory quotient may vary from moment to moment in acute experiments accompanied by changes in alveolar oxygen due to variations in concentration of combustible materials and to variations in the oxidative mechanism.

But assuming a constant true respiratory quotient and subtracting the theoretical oxidative carbon dioxide from the total elimination of carbon dioxide an approximate curve of non-oxidative carbon dioxide elimination was obtained. This curve indicates a large elimination of non-oxidative carbon dioxide during lowered alveolar oxygen and a retention of carbon dioxide during readministration of room air.

Assuming an approximately equal distribution of lactic acid between tissues and blood (the possible fallacy of which is recognised) the total accumulation of lactic acid is computed. This accumulation of lactic acid plus the carbon dioxide formed by oxidation processes greatly exceeds the total formation of acid (oxidative $CO_2$) when oxidations are not impaired. The amount of acid formed exceeds the amount eliminated ($CO_2$).

The quotient of non-oxidative carbon dioxide over the corresponding carbon dioxide equivalent of lactic acid is used as an approximate index to the amount of lactic acid reacting with bicarbonate base. This quotient which is designated as the carbon dioxide index suggests that approximately 40 per cent of the lactic acid which was liberated combined with sodium bicarbonate.

Adding the caloric equivalents of oxygen consumed to the energy shift accompanying the lactic acid shift an approximate curve of energy production was obtained. If the rate of lactic acid production per unit of oxygen shortage is low the curve of energy production falls during a period of lowered alveolar oxygen. If the rate of lactic acid production is high the curve of energy production will rise. The curve of energy production usually fell during anoxemia and invariably rose above the normal level on readministration of room air.

## BIBLIOGRAPHY

GESELL: 1929. Ergebn. d. Physiol., xxviii, 346.
1923. This Journal, lxvi, 5.
1925. Physiol. Rev., v, 551.
1926. Proc. Soc. Exp. Biol. Med., xxiv, 263.
GESELL AND HERTZMAN: 1927. This Journal, lxxxiii, 420.
GESELL, CAPP AND FOOTE. 1922. This Journal, lxiii, 1.

GESELL AND MCGINTY. 1926. This Journal, lxxix, 72.
ROSENCRANZ. 1927. Journ. Optical Soc. of America and Review of Sci. Inst., xiv, 479.
VAN SLYKE AND NEILL. 1924. Journ. Biol. Chem., lxi, 523.
HARRINGTON AND VAN SLYKE. 1924. Journ. Biol. Chem., lxi, 575.
GESELL. 1919. This Journal, xlix, 90.
FRIEDEMANN, COTONIO AND SHAFFER. 1927. Journ. Biol. Chem., lxxiii, 335.
MCGINTY AND GESELL. 1927. This Journal, lxxxiii, 358.
LUSK. 1923. The elements of the science of nutrition. W. B. Saunders Co.
MEYERHOF. 1925. Handbuch der Norm. u. Path. Physiol., viii, 1, 517, 521.
DAVIS AND STATER. 1926. Biochem. Journ., xx, 1167.
1927. Ibid., xxi, 198.
1928. Ibid., xxii, 338.

# THE REGULATION OF RESPIRATION

## A Study of the Correlation of Numerous Factors of Respiratory Control During Intravenous Injection of Sodium Cyanide and Recovery[1, 2]

ROBERT GESELL, HUGO KRUEGER,[3] GRACE GORHAM AND THEODORE BERNTHAL

*From the Department of Physiology, University of Michigan, Ann Arbor*

Received for publication March 17, 1930

In the preceding paper we have analyzed in some detail the effects of anoxemia. In this paper we give attention to the effects of intravenous injection of sodium cyanide. While both anoxemia and sodium cyanide reduce the rate of oxidations the attending conditions are somewhat different and seem to call for comparative study. In the first place the reduction in oxidations is produced in different ways. During anoxemia the blood is poorly oxygenated and the tissues suffer from lack of oxygen. The oxidative mechanism is intact. During cyanide effects the blood is a bright arterial red and is hyperoxygenated. The supply of oxygen is more than adequate, but the oxidative mechanism is impaired and the cells can no longer use oxygen as freely as before. In both conditions the transport of carbon dioxide is impaired by virtue of a broken coördination of the dual function of hemoglobin.[4] This is due to the rapid formation of lactic acid during impaired oxidations and to the absence of adequate reduction of oxyhemoglobin in the tissues to absorb free carbon dioxide and to the absence of adequate oxidation in the lungs to liberate the combined carbon dioxide (Gesell and Hertzman, 1928). During anoxemia the blood is in a relatively constant state of low oxidation and during cyanide poisoning it is in a relatively constant state of high oxidation.

[1] Preliminary report. This Journal, 1929, xc, 359.

[2] This investigation has been made with the assistance of a grant from the Committee on Therapeutic Research, Council on Pharmacy and Chemistry, American Medical Association.

[3] Submitted in partial fulfillment of the requirements for the Degree of Doctor of Philosophy.

[4] While the conception of the "broken coördination of the dual function of hemoglobin" (Gesell) was based on the findings of Haldane, of L. J. Henderson, of Van Slyke and others, the recent findings of Henriques (1929) indicating a direct reaction between carbon dioxide and hemoglobin to form carbohemoglobin do not effect the significance of this conception.

Under the conditions of the present series of experiments in which constant artificial ventilation is administered there is another difference between anoxemia and cyanide poisoning related to the hydrogen ion concentration of the blood. Administration of gaseous mixtures low in oxygen initiates an alkaline change. Administration of cyanide initiates an acid change (Gesell and Hertzman, 1928). With these differences already well established a more detailed study is of interest.

METHOD. On the whole the procedure in the cyanide experiments was the same as in the preceding experiments. Room air was continuously administered at a uniform volume and after optimum conditions were established two sets of blood and gas samples were taken at ten and fifteen minute intervals. Sodium cyanide was then injected intravenously over varying periods of time and the animal allowed to recover. Our objective was to produce relatively severe impairment of oxidations without permanent injury to the tissues. This was difficult. If injection was made too slowly or not carried far enough the effects were too small to follow with sufficient accuracy over short intervals of time. If the injection was rapid and the effects decided, recovery was likely to be imperfect and very slow as compared with recovery on readministration of room air following anoxemia. On the whole the results were not as satisfactory as those on anoxemia where reversibility was more perfect.

RESULTS. *Experiment 1.* The tables and figures show the effects of intravenous injection of sodium cyanide and sodium bicarbonate. The table shows the results of the earlier part of the experiment as well. Preceding the injection of sodium cyanide (not shown in the graph) 150 cc. of saline solution were injected at 1 minute 58 seconds; at 33 minutes 20 seconds the animal was bled 250 cc.; at 39 minutes 18 seconds it was bled 140 cc. more; at 63 minutes 47 seconds the blood was reinjected. Gas samples 8, 9 and 10 follow hemorrhage and immediately precede the injection of sodium cyanide. During this period oxygen consumption and carbon dioxide elimination slowly decreased. There was a slight reduction in the bood lactic acid and a small increase in the blood carbon dioxide capacity. Blood pH and expiratory quotient remained virtually constant. At 110 minutes injection of 0.003M sodium cyanide was begun and ended at 118 minutes. Oxygen absorption and carbon dioxide elimination decreased and the expiratory quotient increased from 0.80 to 1.07. At the end of injection recovery was exceptionally prompt. Gaseous exchange increased rapidly at first, then more slowly and within approximately twenty minutes slowly decreased again. The expiratory quotient dropped rapidly at first to 0.89 and 0.91 respectively then more slowly to 0.84 and then as the condition of the animal deteriorated the expiratory quotient rose once more to 0.90. The results thus agree with the earlier experiments of McGinty and Gesell (1927) on cyanide administration

in which subnormal expiratory quotients of recovery were uncommon. As in the experiments where low oxygen was administered, lactic acid accumulated in the blood during the period of markedly impaired oxidations. During the period of recovery when oxidations increased and returned towards normal, blood lactic acid diminished. There is fair agreement between the curves of carbon dioxide capacity of the blood and blood lactic acid even though the carbon dioxide capacity failed to increase during recovery. The changes in hydrogen ion concentration of the blood agree with the general increase in blood lactic acid and with the general

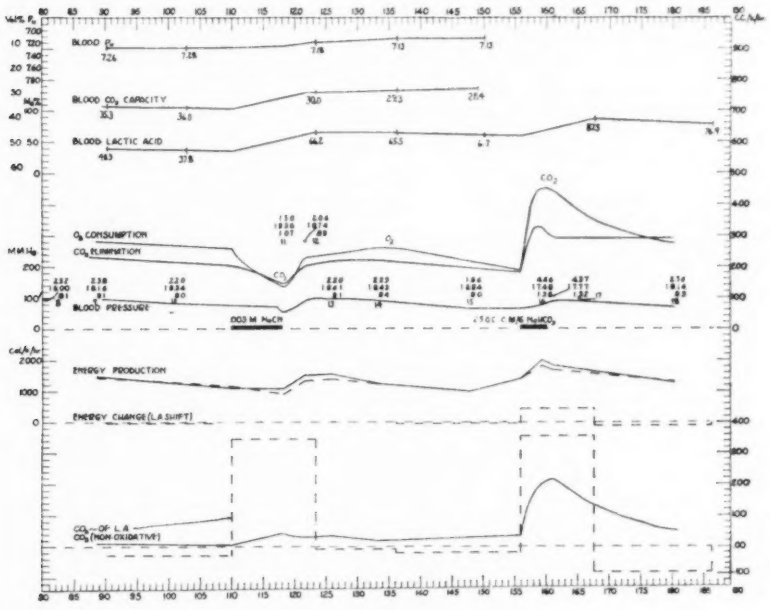



Fig. 1. Experiment 1

decrease in carbon dioxide capacity. The pH findings are similar to findings of earlier experiments in this laboratory (Gesell and Hertzman, 1928).

The gaseous metabolism during recovery differs from the metabolism of recovery following anoxemia in that the expiratory quotient remained above the level of the quotient preceding the injection of cyanide. This difference, however, may be one of degree only and can hardly be taken as evidence of total absence of non-oxidative removal of lactic acid for the drop in the expiratory quotient from 1.07 to 0.89 is relatively rapid. The

TABLE 1

*Experiment 1*

| OBSERVATION | PROCEDURE | TIME | PER CENT OF EXPIRED $CO_2$ | PER CENT OF EXPIRED $O_2$ | EXPIRATORY QUOTIENT | $O_2$ CONSUMPTION IN CC. PER KGM. HR. | $CO_2$ ELIMINATION IN CC. PER KGM. HR. | $CO_2$ ELIMINATION FROM OXIDATIONS BASED ON E.Q. IN CC. PER KGM. HR. | DIFFERENCE BETWEEN ACTUAL AND THEORETICAL $CO_2$ ELIMINATION | $CO_2$ EQUIVALENT OF CHANGE OF LACTIC ACID | LACTIC ACID IN MGM. PER 100 CC. ARTERIAL BLOOD | LACTIC ACID IN MGM. PER 100 CC. VENOUS BLOOD | $CO_2$ CAPACITY OF ARTERIAL BLOOD IN VOLUME PER CENT | $CO_2$ CAPACITY OF VENOUS BLOOD IN VOLUME PER CENT | pH OF ARTERIAL BLOOD | pH OF VENOUS BLOOD | MEAN BLOOD PRESSURE | OXIDATIVE ENERGY IN SMALL CALORIES PER KGM. HR. | NON-OXIDATIVE ENERGY IN SMALL CALORIES PER KGM. HR. | OXIDATIVE + NON-OXIDATIVE ENERGY IN SMALL CALORIES PER KGM. HR. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12A | 12V | 13A | 13V | 14A | 14V | 15 | 16 | 17 | 18 |
| 1 | Saline 150 cc. | 1′ 58″ | | | | | | | | | | | | | | | | | | |
| 2 | G. S. no. 1 | 8′ 28″ | 2.36 | 18.07 | 0.77 | 289.9 | 223.8 | | | | | | | | | | 130 | 1,382 | | |
| 3 | B. S. no. 1 | 12′ 50″ | | | | | | | | | 25.3 | 41.5 | 43.45 | 42.35 | 7.40 | 7.35 | 126 | | | |
| 4 | G. S. no. 2 | 25′ 38″ | 2.33 | 18.10 | 0.77 | 288 | 220.7 | 223.2 | −2 | | | | | | | | 120 | 1,373 | −47 | |
| 5 | B. S. no. 2 | 28′ 28″ | | | | | | | | | 21.3 | 21.2 | 42.9 | 45.9 | 7.41 | 7.36 | | | | |
| 6 | Hem. 250 cc. | 33′ 20″ | | | | | | | | | c 19.7 | c 15 | | | | | 115 | | | |
| 7 | G. S. no. 3 | 35′ 48″ | 2.00 | 18.57 | 0.79 | 239 | 189.2 | 184.5 | +5 | +15 | | | | | | | | 1,139 | +19 | 1,158 |
| 8 | B. S. no. 3A | 37′ 20″ | | | | | | | | | | | | | 7.39 | | 104 | | | |
| 9 | Hem. 140 cc. | 39′ 18″ | | | | | | | | | | | | | | | | | | |
| 10 | G. S. no. 4 | 42′ 33″ | 1.73 | 18.96 | 0.82 | 198.9 | 162.9 | 153 | +10 | +15 | | | | | | | 68 | 953 | +18 | 971 |
| 11 | B. S. no. 3 | 44′ 20″ | | | | | | | | | 20.8 | 19.0 | 42.6 | 4.31 | 7.42 | 7.41 | | | | |
| 12 | G. S. no. 5 | 55′ 13″ | 1.81 | 18.82 | 0.80 | 213.4 | 170.5 | 163 | +8 | +100 | | | | | | | 76 | 1,015 | +119 | 1,134 |
| 13 | B. S. no. 4 | 58′ 14″ | | | | | | | | | 30.1 | 30.9 | 40.1 | 38.0 | 7.39 | 7.39 | | | | |
| 14 | F. V. S. no. 4 | 58′ 55″ | | | | | | | | | | 28.9 | | 39.7 | | 7.33 | | | | • |
| 15 | Inj. 375 cc. blood | 63′ 47″ | | | | | | | | | c 34 | | | | | | 76 | | | |
| 16 | G. S. no. 6 | 65′ 49″ | 3.03 | 17.15 | 0.75 | 384.6 | 286.9 | 297 | −10 | −86 | | | | | | | 117 | 1,833 | −104 | 1,729 |
| 17 | B. S. no. 5A | 65′ 55″ | | | | | | | | | | | | | 7.30 | | | | | |
| 18 | G. S. no. 7 | 70′ 30″ | 2.83 | 17.74 | 0.84 | 319 | 269.2 | 246.2 | +23 | −86 | | | | | | | | 1,520 | −104 | 1,406 |
| 19 | B. S. no. 6 | 72′ 41″ | | | | | | | | | 28.9 | 40.0 | 37.9 | 36.9 | 7.30 | 7.27 | 104 | | | |

| | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 20 | F. V. S. no. 6 | 73′ 19″ | | | | | | | | | | 38.9 | | 37.55 | | 7.25 | | | | |
| 21 | G. S. no. 8 | 80′ 7″ | 2.52 | 18.00 | 0.81 | 306.1 | 242.9 | 231.9 | +11 | +97 | | | | | | | 103 | 1,430 | +117 | 1,547 |
| 22 | G. S. no. 9 | 88′ 30″ | 2.38 | 18.16 | 0.81 | 283.3 | 229.1 | 218.8 | +9 | +97 | | | | | | | | 1,349 | +117 | 1,466 |
| 23 | B. S. no. 7 | 90′ 15″ | | | | | | | | | 40.3 | 38.4 | 31.4 | 35.3 | 7.30 | 7.26 | 97 | | | |
| 24 | F. V. S. no. 7 | 90′ 30″ | | | | | | | | | | 44.7 | | 38.6 | | 7.28 | | | | |
| 25 | G. S. no. 10 | 100′ 55″ | 2.20 | 18.34 | 0.80 | 269.2 | 214.4 | 208 | +6 | −30 | | | | | | | | 1,282 | −35 | 1,247 |
| 26 | B. S. no. 8 | 102′ 0″ | | | | | | | | | 37.8 | 49.4 | | 36.0 | 7.28 | | 80 | | | |
| 27 | NaCn 0.0033 N | 110′ | | | | | | | | | c 36 | | | | | | 76 | | | |
| 28 | G. S. no. 11 | 118′ 18″ | 1.50 | 19.56 | 1.07 | 135.7 | 145.4 | 104.7 | +41 | +344 | | | | | | | 54 | 646 | +416 | 1,062 |
| 29 | G. S. no. 12 | 121′ 40″ | 2.06 | 18.74 | 0.89 | 227.7 | 202 6 | 175.8 | +28 | +34 | | | | | | | 88 | 1,085 | +416 | 1,501 |
| 30 | B. S. no. 9 | 121′ 55″ | | | | | | | | | 66.2 | 59.6 | | 30.0 | 7.18 | | | | | |
| 31 | G. S. no. 13 | 125′ 58″ | 2.20 | 18.61 | 0.91 | 239.6 | 218.2 | 185 | +33 | −8 | | | | | | | 96 | 1,140 | +416 | 1,556 |
| 32 | G. S. no. 14 | 133′ 20″ | 2.23 | 18.43 | 0.84 | 261.7 | 220.4 | 202 | +18 | −8 | | | | | | | 90 | 1,247 | −9 | 1,238 |
| 33 | B. S. no. 10 | 134′ 50″ | | | | | | | | | 65.5 | 62.5 | | 29.25 | 7.13 | | | | | |
| 34 | G. S. no. 15 | 148′ 0″ | 1.96 | 18.84 | 0.90 | 216.5 | 194.3 | 167.3 | +27 | −41 | | | | | | | 64 | 1,032 | −52 | 980 |
| 35 | B. S. no. 11 | 149′ 10″ | | | | | | | | | 61.7 | 70.6 | | 28.4 | 7.13 | | | | | |
| 36 | $NaHCO_3$ 250 cc. | 156′ 3″ | | | | | | | | | | | | | | | 64 | | | |
| 37 | G. S. no. 16 | 159′ 20″ | 4.46 | 17.48 | 1.38 | 329 | 453.3 | 254 | 200 | +356 | | | | | | | 76 | 1,568 | +433 | 2,001 |
| 38 | G. S. no. 17 | 161′ 3″ | 4.37 | 17.77 | 1.52 | 293.8 | 445.3 | 227 | 218 | | | | | | | | 88 | 1,400 | +433 | 1,833 |
| 39 | B. S. no. 12 | 167′ 38″ | | | | | | | | | 87.5 | | | | | | 85 | | | |
| 40 | G. S. no. 18 | 180′ 28″ | 2.76 | 18.14 | 0.95 | 290.2 | 275.3 | 224 | 51 | −86 | | | | | | | 69 | 1,384 | +120 | 1,264 |
| 41 | B. S. no. 13 | 186′ 20″ | | | | | | | | | 76.9 | | | | | | | | | |

absence of a further drop is to be attributed to a less complete recovery of the tissues and to a partial impairment of the non oxidative mechanism of removal of lactic acid.

Considering next the elimination of non-oxidative carbon dioxide it will be seen from the graph that this is exceedingly small, particularly when compared with the theoretical maximum liberated by the reaction of the newly formed lactic acid with bicarbonate. According to table 6 the carbon dioxide index from the beginning of injection of sodium cyanide to gas sample 11 is only 0.08; from gas sample 11 to gas sample 12 it is 0.12; from gas sample 12 to gas sample 13 it is 0.19 and from the injection of sodium cyanide to gas sample 15 the carbon dioxide index is 0.24. These figures stand in striking contrast to the higher indices of the low alveolar oxygen experiments. Especially does this hold for the period of rapid formation of lactic acid when during anoxemia the elimination of non-oxidative carbon dioxide is usually very high. The absence of absolute retention of carbon dioxide following the end of injection as shown in the graph is also in contrast with the usual results following readministration of room air after anoxemia. The curve of energy production will be seen to rise. This is due to the high rate of formation of lactic acid with respect to oxidation shortage.

*Experiment 2.* Sodium cyanide was injected early in the experiment and gas samples 1 and 2 were taken shortly before injection. During this pre-administration period oxygen absorption and carbon dioxide elimination and the changes in blood pH, blood carbon dioxide capacity and blood lactic acid were very small and negligible for purposes of comparison with the changes occurring during the subsequent period of injection. At 27 minutes 23 seconds sodium cyanide was injected. At gas sample 3 oxygen absorption had fallen from 493 cc. per kgm. hour to 423.6 cc. and carbon dioxide elimination had risen from 380.2 cc. per kgm. hour to 386.9 cc. The expiratory quotient rose from 0.77 to 0.91. This initial increased elimination of carbon dioxide during the period of impaired oxidations while differing from experiment 1 of this series resembles results of some of the anoxemia experiments. From gas sample 3 to gas sample 4 both oxygen absorption and carbon dioxide elimination decrease, but as oxygen consumption falls more rapidly the expiratory quotient increases from 0.91 to 0.97. This period of impaired oxidation was accompanied by the usual increase in blood lactic acid and blood acidity and decrease in the blood carbon dioxide capacity. At the end of injection of cyanide there was a distinct increase in gaseous metabolism but the maximum elimination of carbon dioxide and maximum absorption of oxygen failed to reach the initial normal level. In agreement with this permanent shortage of oxidations blood lactic acid and blood acidity continued to increase, but rather strangely the carbon dioxide capacity of the blood showed

only a minor decrease. While oxygen absorption and carbon dioxide elimination following injection of sodium cyanide failed to reach the initial level the shape of the curves suggest overshooting common to so many of the experiments on the effects of lowered alveolar oxygen. Construing this portion of the curves as indicative of overshooting this overshooting must be looked upon as being superimposed on a lower level of gaseous metabolism. The drop in the expiratory quotient from 0.97 to 0.92 and 0.87 with a subsequent rise may be construed in favor of a temporary non-oxidative removal of carbon dioxide.

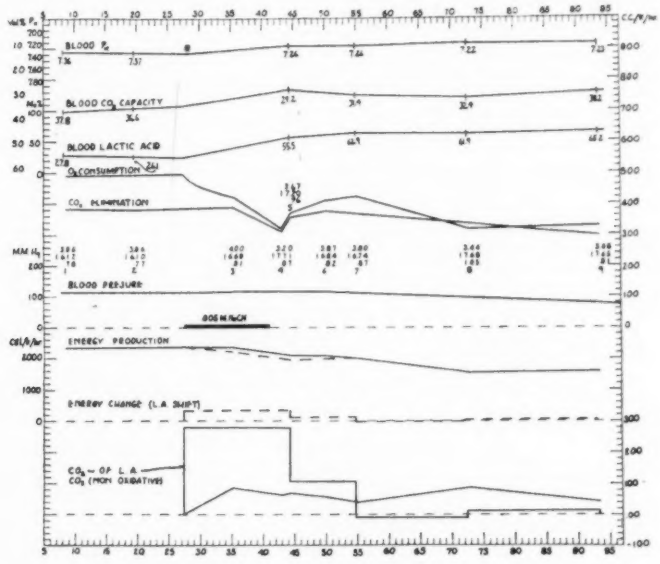



Fig. 2. Experiment 2

Comparing the areas of the carbon dioxide equivalent of lactic acid and the non-oxidative carbon dioxide, the relatively small liberation of non-oxidative carbon dioxide during the early period of impaired oxidation is indicated again. From the injection of sodium cyanide to gas sample 4 the carbon dioxide index is only 0.24 but as the period becomes more inclusive the index rises. From the period between injection and gas sample 8 it is 0.50 and for the period between injection and gas sample 9 it has reached a relatively high figure 0.68. At no point was there absolute retention of carbon dioxide.

During the early part of the period of impaired oxidations the energy liberated by the formation of lactic acid more than compensates for the

TABLE 2

*Experiment 2*

| OBSERVATION | PROCEDURE | TIME | PER CENT OF EXPIRED $CO_2$ | PER CENT OF EXPIRED $O_2$ | EXPIRATORY QUOTIENT | $O_2$ CONSUMPTION IN CC. PER KGM. HR. | $CO_2$ ELIMINATION IN CC. PER KGM. HR. | $CO_2$ ELIMINATION FROM OXIDATIONS BASED ON E.Q. IN CC. PER KGM. HR. | DIFFERENCE BETWEEN ACTUAL AND THEORETICAL $CO_2$ ELIMINATION | $CO_2$ EQUIVALENT OF CHANGE OF LACTIC ACID | LACTIC ACID IN MGM. PER 100 CC. ARTERIAL BLOOD | LACTIC ACID IN MGM. PER 100 CC. VENOUS BLOOD | $CO_2$ CAPACITY OF ARTERIAL BLOOD IN VOLUME PER CENT | $CO_2$ CAPACITY OF VENOUS BLOOD IN VOLUME PER CENT | pH OF ARTERIAL BLOOD | pH OF VENOUS BLOOD | MEAN BLOOD PRESSURE | OXIDATIVE ENERGY IN SMALL CALORIES PER KGM. HR. | NON-OXIDATIVE ENERGY IN SMALL CALORIES PER KGM. HR. | OXIDATIVE + NON-OXIDATIVE ENERGY IN SMALL CALORIES PER KGM. HR. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12A | 12V | 13A | 13V | 14A | 14V | 15 | 16 | 17 | 18 |
| 1 | G. S. no. 1 | 8′ 26″ | 3.96 | 16.12 | 0.78 | 490.6 | 380.8 | | | | | | | | | | | 2,340 | | |
| 2 | A. S. no. 1 | 8′ 10″ | | | | | | | | | 27.8 | | 37.8 | | 7.36 | | 115 | | | |
| 3 | V. S. no. 1 | 9′ 30″ | | | | | | | | | | 35.0 | | 36.27 | | 7.35 | | | | |
| 4 | G. S. no. 2 | 19′ 30″ | 3.96 | 16.10 | 0.77 | 493.0 | 380.2 | | | | | | | | | | 114 | 2,350 | −27 | |
| 5 | A. S. no. 2 | 19′ 30″ | | | | | | | | | 26.1 | | 36.6 | | 7.37 | | | | | |
| 6 | V. S. no. 2 | 20′ 13″ | | | | | | | | | | 38.2 | | 37.3 | | 7.34 | | | | |
| 7 | Inj. NaCN 0.005 m | 27′ 23″ | | | | | | | | | c 24.0 | | | | | | | | | |
| 8 | G. S. no. 3 | 35′ 18″ | 4.00 | 16.69 | 0.91 | 423.6 | 386.9 | 300 | 87 | +279 | | | | | | | 116 | 2,020 | +338 | 2,358 |
| 9 | Inj. NaCN 0.005 m | 37′ 32″ | | | | | | | | | | | | | | | | | | |
| 10 | G. S. no. 4 | 43′ | 3.20 | 17.71 | 0.97 | 318.6 | 310.0 | 247 | 63 | +279 | | | | | | | | 1,520 | +338 | 1,858 |
| 11 | A. S. no. 3 | 44′ 20″ | | | | | | | | | 55.5 | | 29.2 | | 7.26 | | | | | |
| 12 | G. S. no. 5 | 44′ 25″ | 3.67 | 17.20 | 0.96 | 369.0 | 356.1 | 286 | 70 | +279 | | | | | | | | 1,760 | +338 | 2,098 |
| 13 | V. S. no. 3 | 45′ 25″ | | | | | | | | | | 60.3 | | 35.5 | | 7.25 | | | | |
| 14 | G. S. no. 6 | 50′ | 3.87 | 16.84 | 0.92 | 408 | 374.2 | 316.8 | 58 | +108 | | | | | | | 115 | 1,950 | +131 | 2,081 |
| 15 | A. S. no. 4 | 54′ 51″ | | | | | | | | | 62.9 | | 31.35 | | 7.26 | | | | | |
| 16 | G. S. no. 7 | 55′ 6″ | 3.80 | 16.74 | 0.87 | 421.4 | 367.3 | 327 | 40 | −9 | | | | | | | | 2,010 | −10 | 2,000 |
| 17 | V. S. no. 4 | 56′ 6″ | | | | | | | | | | 66.0 | | 29.4 | | 7.24 | | | | |

| | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 19 | A. S. no. 5 | 72′ 25″ | | | | | | | | | 61.9 | | 32.4 | | 7.22 | | 95 periodic | | | | |
| 20 | G. S. no. 8 | 72′ 50″ | 3.44 | 17.68 | 1.05 | 317.3 | 334.0 | 246 | 88 | +23 | | | | | | | | 1,520 | +27 | 1,547 |
| 21 | V. S. no. 5 | 73′ 45″ | | | | | | | | | | 67.0 | | 29.0 | | 7.21 | | | | |
| 22 | A. S. no. 6 | 93′ 19″ | | | | | | | | | 65.2 | | 30.2 | | 7.23 | | 80 | | | | |
| 23 | G. S. no. 9 | 93′ 35″ | 3.08 | 17.65 | 0.91 | 328.4 | 297.4 | 255 | 42 | +23 | | | | | | | | 1,570 | +27 | 1,597 |
| 24 | V. S. no. 6 | 94′ 50″ | | | | | | | | | | 68.0 | | 30.7 | | 7.20 | | | | |
| 25 | R. ② | 106′ | | | | | | | | | | | | | | | | | | |
| 26 | A. S. no. 7 | 112′ 5″ | | | | | | | | | 66.8 | | 32.6 | | 7.23 | | 74 periodic | | | | |
| 27 | G. S. no. 10 | 112′ 15″ | 3.03 | 17.76 | 0.92 | 316.9 | 292.5 | 246 | 46 | +13 | | | | | | | | 1,510 | +17 | 1,527 |
| 28 | V. S. no. 7 | 113′ 25″ | | | | | | | | | | 68.1 | | 33.8 | | 7.17 | | | | |
| 29 | m/$NaHCO_3$, 425 cc. | 121′ | | | | | | | | | c 67.6 | c 68.1 | | | | | 76 | | | | |
| 30 | G. S. no. 11 | 123′ 10″ | 5.23 | 16.86 | 1.37 | 377.6 | 516.5 | 293 | 224 | +616 | | | | | | | 95 | 1,790 | +720 | 2,510 |
| 31 | G. S. no. 12 | 127′ 38″ | 6.55 | 16.14 | 1.49 | 437.9 | 652.6 | 339 | 314 | +612 | | | | | | | 100 | 2,080 | +720 | 2,800 |
| 32 | End of inj. | 129′ 3″ | | | | | | | | | | | | | | | | | | |
| 33 | A. S. no. 8 | 130′ 51″ | | | | | | | | | 107.8 | | 130.2 | | 7.63 | | | | | |
| 34 | V. S. no. 8 | 130′ 43″ | | | | | | | | | | 110.0 | | 121.3 | | 7.57 | | | | |
| 35 | G. S. no. 13 | 130′ 48″ | 5.91 | 16.35 | 1.35 | 434.1 | 585.3 | 336.8 | 248 | +612 | | | | | | | 84 | 2,070 | +720 | 2,790 |
| 36 | G. S. no. 14 | 144′ 41″ | 4.39 | 16.96 | 1.12 | 383.4 | 428.6 | 297.6 | 131 | +162 | | | | | | | 68 | 1,830 | +195 | 2,015 |
| 37 | A. S. no. 9 | 145′ 22″ | | | | | | | | | 123.3 | | 83.1 | | 7.55 | | | | | |
| 38 | V. S. no. 9 | 146′ | | | | | | | | | | 127.5 | | 87.4 | | 7.51 | | | | |
| 39 | G. S. no. 15 | 169′ | 3.71 | 17.82 | 1.23 | 294.1 | 362.7 | 228 | 135 | +186 | | | | | | | 45 | 1,400 | +223 | 1,623 |
| 40 | V. S. no. 10 | 171′ 20″ | | | | | | | | | | 155.5 | | 66.2 | | | 0 | | | |
| 41 | A. S. no. 10 | 171′ 35″ | | | | | | | | | 149.9 | | 68.55 | | | | | | | |

energy lost by reduction in oxidations. Energy production rose from gas sample 2 to gas sample 3 from 1858 calories per kgm. hour to 2098. Corresponding to this rise there was a small oxygen lactic acid ratio of 40. From this point on the ratio is relatively large. From gas sample 3 to gas sample 4 it is 139; from sample 4 to 5 it is 141; from sample 5 to 6 it is 282; and from sample 6 to 7 it is 168; from sample 8 to 9 it is 179. There was consequently a continued fall in energy production despite increasing oxygen absorption from gas sample 4 to 5 to 6 to 7.

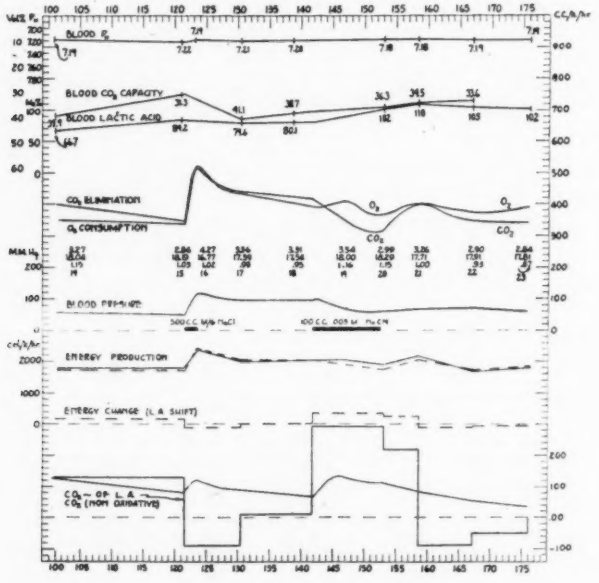



Fig. 3. Experiment 3

*Experiment 3.* Sodium cyanide was injected very late in the course of this experiment—at 141 minutes 50 seconds. The preceding history of the experiment is—administration of 11.46 per cent $CO_2$ in 21.39 per cent $O_2$ at 19 minutes 58 seconds, room air at 34 minutes 58 seconds, 19.22 per cent $CO_2$ in 27.12 per cent $O_2$ at 64 minutes 1 second, room air 81 minutes 50 seconds, and 500 cc. saline at 121 minutes 5 seconds. We have, therefore, to consider not only a generally deteriorated condition of the animal but the effects of previous administration of carbon dioxide and saline solution. The elimination of carbon dioxide which was absorbed and stored during the administration of carbon dioxide must be differentiated from the elimination of the true oxidative carbon dioxide. Pre-

ceding the injection of saline solution oxygen consumption was nearly constant—349.9 cc. per kgm. hour at gas sample 14 and 339.5 cc. at gas sample 15. Carbon dioxide elimination 400.8 and 348 cc. per kgm. hour respectively was in excess of oxygen absorption. At gas sample 14 the expiratory quotient was 1.15 and at gas sample 15 it was 1.03. Since lactic acid was increasing between these samples it must be considered as one source of the excess carbon dioxide. The general downward gradient of the curve of non-oxidative carbon dioxide indicates that previously stored carbon dioxide is another source. When sodium chloride was administered oxygen absorption and carbon dioxide elimination increased to approximately the same extent. The elimination of carbon dioxide fell off more rapidly than absorption of oxygen and the expiratory quotient fell from 1.02 to 0.99 to 0.95. The elimination of non-oxidative carbon dioxide continued to fall along a similar gradient.

At 141 minutes 50 seconds 100 cc. 0.003M NaCN were injected over a period of ten minutes. Between gas samples 18 and 19 oxygen absorption fell off from 423.8 to 353.2 cc. per kgm. hour and carbon dioxide elimination increased from 403.4 to 409.6 cc. per kgm. hour with an increase in the expiratory quotient from 0.95 to 1.16. Between gas samples 19 and 20 both oxygen absorption and carbon dioxide elimination decreased and the expiratory quotient remained at 1.15. During recovery oxygen absorption increased more rapidly than elimination of carbon dioxide. The curves cross and the expiratory quotient falls considerably below unity. These results resemble some of those with the administration of low oxygen, in that carbon dioxide elimination actually increases though oxygen absorption falls off and in that the curves recross during recovery. These changes in gaseous metabolism were accompanied by increase and decrease in blood lactic acid. The carbon dioxide index, however, is again distinctly low during impaired oxidations as compared with the low oxygen experiments. These indices have been computed in two ways—one correcting for the gradient and the other ignoring the gradient of the curve of elimination of non-oxidative carbon dioxide. The computations correcting for the non-oxidative carbon dioxide would appear to be the more reliable. According to these values the carbon dioxide index for the period of increasing blood lactic acid was 0.25. For the more inclusive period from the beginning of injection to gas sample 23 which takes in the period of disappearance of blood lactic acid the figure of 0.52 approaches the more common value for the low oxygen experiments.

The curve of energy production rises and falls in accordance with the changes in gaseous metabolism and the lactic acid shift. At gas sample 21 it is higher than at gas sample 18 immediately preceding the injection of sodium cyanide, namely, 2170 calories per kgm. hour as compared with 2031.

TABLE 3

*Experiment 3*

| OBSERVATION | PROCEDURE | TIME | PER CENT OF EXPIRED $CO_2$ | PER CENT OF EXPIRED $O_2$ | EXPIRATORY QUOTIENT | $O_2$ CONSUMPTION IN CC. PER KGM. HR. | $CO_2$ ELIMINATION IN CC. PER KGM. HR. | $CO_2$ ELIMINATION FROM OXIDATIONS BASED ON E.Q. IN CC. PER KGM. HR. | DIFFERENCE BETWEEN ACTUAL AND THEORETICAL $CO_2$ ELIMINATION | $CO_2$ EQUIVALENT OF CHANGE OF LACTIC ACID | LACTIC ACID IN MGM. PER 100 CC. ARTERIAL BLOOD | $CO_2$ CAPACITY OF ARTERIAL BLOOD IN VOLUME PER CENT | pH OF ARTERIAL BLOOD | MEAN BLOOD PRESSURE | OXIDATIVE ENERGY IN SMALL CALORIES PER KGM. HR. | NON-OXIDATIVE ENERGY IN SMALL CALORIES PER KGM. HR. | OXIDATIVE + NON-OXIDATIVE ENERGY IN SMALL CALORIES PER KGM. HR. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 |
| 1 | G. S. no. 1 | 6′ 18″ | 3.24 | 17.03 | 0.78 | 498.4 | 389.6 | 390 | | | | | | 118 | 2,378 | | |
| 2 | A. S. no. 1 | 6′ 27″ | | | | | | | | | 39.3 | 52.20 | 7.34 | | | | |
| 3 | G. S. no. 2 | 15′ 15″ | 3.25 | 17.04 | 0.79 | 497.2 | 390.2 | 389 | | | | | | 110 | 2,371 | | |
| 4 | A. S. no. 2 | 15′ 23″ | | | | | | | | | 40.0 | 49.41 | 7.41 | | | | |
| 5 | 11.46% $CO_2$, 21.396% $O_2$ | 19′ 58″ | | | | | | | | | c 40.3 | | | 106 | | | |
| 6 | G. S. no. 3 | 26′ 41″ | 12.82 | 18.58 | 0.26 | 435.0 | 111 | 340.2 | −229 | | | | | 117 | 2,073 | +21 | 2,093 |
| 7 | A. S. no. 3 | 26′ 48″ | | | | | | | | | 41.1 | 48.12 | 7.20 | | | | |
| 8 | G. S. no. 4 | 33′ 42″ | 13.18 | 18.37 | 0.35 | 455.9 | 158.3 | 356.2 | −198 | | | | | 123 | 2,172 | +254 | 2,426 |
| 9 | A. S. no. 4 | 33′ 50″ | | | | | | | | | 50.9 | 46.38 | 7.19 | | | | |
| 10 | Room air | 34′ 58″ | | | | | | | | | | | | | | | |
| 11 | G. S. no. 5 | 39′ 23″ | 4.67 | 16.28 | 0.99 | 574.6 | 569.1 | 450.5 | +119 | | | | | 95 | 2,740 | +251 | 2,991 |
| 12 | A. S. no. 5 | 39′ 35″ | | | | | | | | | 58.8 | 44.10 | 7.25 | | | | |
| 13 | G. S. no. 6 | 45′ 51″ | 3.96 | 17.21 | 1.06 | 454 | 483 | 355 | +128 | | | | | 87 | 2,163 | +19 | 2,182 |
| 14 | A. S. no. 6 | 46′ 12″ | | | | | | | | | 59.5 | 41.42 | 7.25 | | | | |
| 15 | G. S. no. 7 | 58′ 32″ | 3.29 | 17.46 | 0.91 | 435.1 | 397.7 | 340 | +58 | | | | | 85 | 2,075 | +79 | 2,154 |
| 16 | A. S. no. 7 | 58′ 57″ | | | | | | | | | 65.0 | 44.39 | 7.31 | | | | |
| 17 | 19.22% $CO_2$, 27.125% $O_2$ | 64′ 1″ | | | | | | | | | c 67.2 | | | 84 | | | |
| 18 | G. S. no. 8, $O_2$ only | 70′ 40″ | | 24.66 | | 424.3 | | 332 | −371 | | | | | 110 | 2,022 | −63 | 1,959 |
| 19 | G. S. no. 9 $CO_2$ | 71′ 59″ | 19.60 | | 0.99 | | | −39 | | | | | | 107 | | | |

| | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 19 | G. S. no. 9, $CO_2$ only | 71′ 59″ | 19.60 | | 0.09 | | −39 | | | | | | | 107 | | | |
| 20 | A. S. no. 8 | 72′ 9″ | | | | | | | | | 64.4 | 41.48 | 7.17 | | | | |
| 21 | G. S. no. 10, $O_2$ only | 79′ 0″ | | 24.69 | | 358.1 | | 280 | −136 | | | | | 118 | 1,708 | −136 | 1,572 |
| 22 | G. S. no. 11, $CO_2$ only | 80′ 36″ | 20.72 | | 0.40 | | 144.0 | | | | | | | 112 | | | |
| 23 | A. S. no. 9 | 80′ 36″ | | | | | | | | | 58.1 | 39.82 | 7.03 | | | | |
| 24 | Room air | 81′ 50″ | | | | | | | | | | | | | | | |
| 25 | G. S. no. 12 | 86′ 4″ | 4.38 | 17.70 | 1.47 | 369.6 | 543.2 | 289 | +254 | | | | | 73 | 1,762 | +59 | 1,821 |
| 26 | A. S. no. 10 | 86′ 10″ | | | | | | | | | 59.9 | 42.10 | 7.08 | | | | |
| 27 | G. S. no. 13 | 93′ 45″ | 3.57 | 17.96 | 1.23 | 356.0 | 438.6 | 278.3 | +160 | | | | | 70 | 1,698 | +55 | 1,753 |
| 28 | A. S. no. 11 | 93′ 53″ | | | | | | | | | 62.2 | 41.90 | 7.23 | | | | |
| 29 | G. S. no. 14 | 100′ 38″ | 3.27 | 18.04 | 1.15 | 349.9 | 400.8 | 273.5 | +127 | +97 | | | | 60 | 1,669 | +117 | 1,786 |
| 30 | A. S. no. 12 | 100′ 53″ | | | | | | | | | 66.7 | 39.85 | 7.19 | | | | |
| 33 | G. S. no. 15 | 120′ 48″ | 2.86 | 18.19 | 1.03 | 339.5 | 348.8 | 265.5 | +83 | +130 | | | | 50 | 1,619 | +158 | 1,777 |
| 34 | A. S. no. 13 | 121′ 5″ | | | | | | | | | 84.2 | 31.26 | 7.22 | | | | |
| 35 | Saline 500 cc. | 121′ 38″ | | | | | | | | | c 84.9 | | | 50 | | | |
| 36 | G. S. no. 16 | 123′ 33″ | 4.27 | 16.77 | 1.02 | 514.7 | 523.4 | 403.7 | 120 | −89 | | | | 115 | 2,452 | −102 | 2,344 |
| 37 | A. S. no. 13A | 123′ 23″ | | | | | | | | | | | 7.19 | | | | |
| 38 | G. S. no. 17 | 130′ 23″ | 3.56 | 17.39 | 0.99 | 441.1 | 434.9 | 345 | 90 | −89 | | | | 95 | 2,100 | −108 | 1,992 |
| 39 | A. S. no. 14 | 130′ 30″ | | | | | | | | | 79.6 | 41.05 | 7.21 | | | | |
| 40 | G. S. no. 18 | 138′ 42″ | 3.31 | 17.54 | 0.95 | 423.8 | 403.4 | 331.3 | 72 | +9 | | | | 95 | 2,020 | +11 | 2,031 |
| 41 | A. S. no. 15 | 138′ 47″ | | | | | | | | | 80.1 | 38.68 | 7.20 | | | | |
| 42 | 100 cc. NaCN | 141′ 50″ | | | | | | | | | c 80.3 | | | 95 | | | |
| 43 | G. S. no. 19 | 146′ 38″ | 3.34 | 18.00 | 1.16 | 353.2 | 409.6 | 276.2 | 133 | +292 | | | | 70 | 1,684 | +355 | 2,039 |
| 44 | G. S. no. 20 | 152′ 55″ | 2.99 | 18.29 | 1.15 | 319.3 | 366 | 249.8 | +116 | +228 | | | | 60 | 1,522 | +355 | 1,877 |
| 45 | A. S. no. 16 | 153′ 5″ | | | | | | | | | 102 | 36.31 | 7.18 | | 1,522 | | |
| 46 | G. S. no. 21 | 158′ 33″ | 3.26 | 17.71 | 1.00 | 399.8 | 398.6 | 312.5 | 86 | +218 | | | | 67 | 1,905 | +265 | 2,170 |
| 47 | A. S. no. 17 | 158′ 35″ | | | | | | | | | 110 | 34.51 | 7.18 | | | | |
| 48 | G. S. no. 22 | 167′ 5″ | 2.90 | 17.91 | 0.93 | 373.4 | 346.7 | 292 | 55 | −88 | | | | 70 | 1,780 | −107 | 1,672 |
| 49 | A. S. no. 18 | 167′ 5″ | | | | | | | | | 105 | 33.56 | 7.19 | | | | |
| 50 | G. S. no. 23 | 176′ 5″ | 2.84 | 17.81 | 0.87 | 389.6 | 338.8 | 304.6 | 34 | −50 | | | | 58 | 1,858 | −60 | 1,798 |
| 51 | A. S. no. 19 | 176′ 11″ | | | | | | | | | 102 | No sample | 7.19 | | | | |

*Experiment 4.* Sodium cyanide was injected at 100 minutes 50 seconds. Previous to this 11.5 per cent $CO_2$ in a 21.2 per cent $O_2$ mixture was administered at 21 minutes 54 seconds, room air readministered at 33 minutes 48 seconds, and 200 cc. saline injected at 55 minutes. Between gas samples 8 and 9 preceding cyanide injection, oxygen consumption and carbon dioxide elimination fell off rapidly yet blood lactic acid and blood carbon dioxide capacity remained nearly constant. The change in blood pH, however, is slightly in the acid direction. The fall in the expiratory quotient can be explained by the decreasing elimination of carbon dioxide

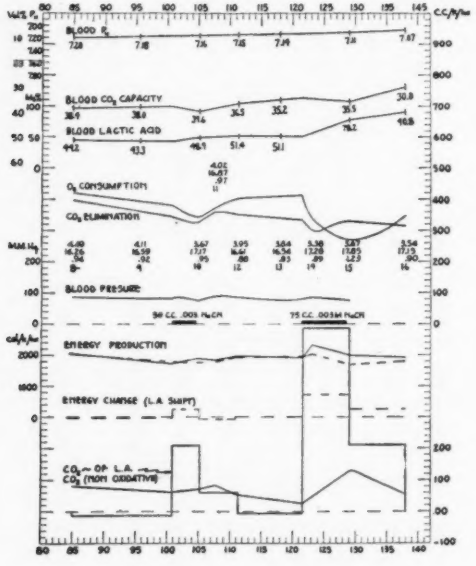



Fig. 4. Experiment 4

previously absorbed during the administration of carbon dioxide. At 100 minutes 50 seconds 50 cc. of 0.003M NaCn were injected. Oxygen absorption and carbon dioxide elimination decreased, the expiratory quotient increased, blood lactic acid and blood acidity increased. Blood carbon dioxide capacity according to analysis increased. This is counter to the regular run of results but since this finding is repeated on the second administration it is rather difficult to attribute it to accidental error in analysis. With recovery both oxygen absorption and carbon dioxide elimination run higher than the initial rates preceding injection. The curves diverge with a resulting drop in the expiratory quotient to 0.83 indicating that some lactic acid is being removed by non-oxidative pro-

TABLE 4

*Experiment 4*

| OBSERVATION | PROCEDURE | TIME | PER CENT OF EXPIRED $CO_2$ | PER CENT OF EXPIRED $O_2$ | EXPIRATORY QUOTIENT | $O_2$ CONSUMPTION IN CC. PER KGM. HR. | $CO_2$ ELIMINATION IN CC. PER KGM. HR. | $CO_2$ ELIMINATION FROM OXIDATIONS BASED ON E.Q. IN CC. PER KGM. HR. | DIFFERENCE BETWEEN ACTUAL AND THEORETICAL $CO_2$ ELIMINATION | $CO_2$ EQUIVALENT OF CHANGE OF LACTIC ACID | LACTIC ACID IN MGM. PER 100 CC. ARTERIAL BLOOD | $CO_2$ CAPACITY OF ARTERIAL BLOOD IN VOLUME PER CENT | pH OF ARTERIAL BLOOD | MEAN BLOOD PRESSURE | OXIDATIVE ENERGY IN SMALL CALORIES PER KGM. HR. | NON-OXIDATIVE ENERGY IN SMALL CALORIES PER KGM. HR. | OXIDATIVE + NON-OXIDATIVE ENERGY IN SMALL CALORIES PER KGM. HR. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 |
| 18 | G. S. no. 8 | 84′ 53″ | 4.49 | 16.26 | 0.94 | 422.2 | 396.4 | 315.8 | 81 | +38 | | | | 86 | 2,000 | +46 | 2,046 |
| 19 | A. S. no. 7 | 84′ 57″ | | | | | | | | | 44.2 | 38.42 | 7.20 | | | | |
| 20 | G. S. no. 9 | 95′ 28″ | 4.11 | 16.59 | 0.92 | 393.9 | 362.3 | 294.2 | +68 | −12 | | | | 82 | 1,865 | −15 | 1,850 |
| 21 | A. S. no. 8 | 95′ 35″ | | | | | | | | | 43.3 | 37.96 | 7.18 | | | | |
| 22 | 50 cc. 0.0033 m NaCN | 100′ 50″ | | | | | | | | | c 42.9 | | | 82 | | | |
| 23 | G. S. no. 10 | 104′ 53″ | 3.67 | 17.17 | 0.95 | 344.2 | 327.6 | 257.2 | +70 | +209 | | | | 76 | 1,630 | +255 | 1,885 |
| 24 | A. S. no. 9 | 105′ 8″ | | | | | | | | | 48.9 | 39.58 | 7.16 | | | | |
| 25 | G. S. no. 11 | 107′ 37″ | 4.02 | 16.87 | 0.97 | 370.2 | 359.4 | 276.7 | +83 | +60 | | | | 88 | 1,752 | +73 | 1,826 |
| 26 | A. S. no. 9A | 107′ 35″ | | | | | | | | | | | 7.16 | | | | |
| 27 | G. S. no. 12 | 111′ 7″ | 3.95 | 16.61 | 0.88 | 399.3 | 351.6 | 298.3 | +53 | +60 | | | | 88 | 1,890 | +73 | 1,963 |
| 28 | A. S. no. 10 | 111′ 20″ | | | | | | | | | 51.4 | 36.53 | 7.15 | | | | |
| 29 | G. S. no. 13 | 117′ 57″ | 3.84 | 16.54 | 0.83 | 409.5 | 340.9 | 306 | 35 | −7 | | | | 78 | 1,939 | +8 | 1,931 |
| 30 | A. S. no. 11 | 118′ 1″ | | | | | | | | | 51.1 | 35.17 | 7.14 | | | | |
| 31 | 75 cc. 0.0033 m NaCN | 121′ 33″ | | | | | | | | | c 51.0 | | | 80 | | | |
| 32 | G. S. no. 14 | 123′ 9″ | 3.38 | 17.28 | 0.89 | 337.2 | 300.7 | 252 | 49 | +587 | | | | 86 | 1,596 | +720 | 2,316 |
| 33 | G. S. no. 15 | 129′ 3″ | 3.67 | 17.85 | 1.23 | 268.5 | 329.9 | 200.8 | 129 | +587 | | | | 77 | 1,271 | +720 | 1,991 |
| 34 | A. S. no. 12 | 129′ 3″ | | | | | | | | | 78.2 | 35.53 | 7.11 | | | | |
| 36 | G. S. no. 16 | 138′ | 3.54 | 17.13 | 0.90 | 348.9 | 315.1 | 260.7 | 55 | +211 | | | | | 1,651 | +257 | 1,908 |
| 37 | A. S. no. 13 | 138′ | | | | | | | | | 90.8 | 29.98 | 7.07 | | | | |

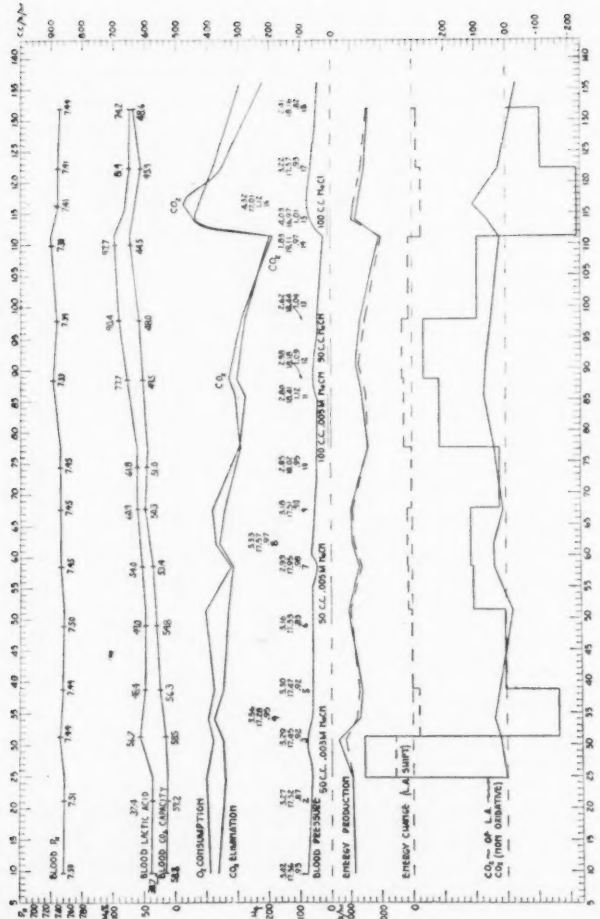



Fig. 5. Experiment 5

cesses though the curve of blood lactic acid shows only a slight reduction in lactic acid. The second and larger injection of cyanide produced more decided effects. Oxygen absorption fell off through gas samples 14 and 15 while carbon dioxide elimination fell off only temporarily and then began to rise during the rapid formation of lactic acid. The expiratory quotient was correspondingly larger than during the first injection of cyanide. As oxidation tended towards recovery and lactic acid accumulated at a slower rate the expiratory quotient falls again below unity. Once more there is disagreement between the curves of blood carbon dioxide capacity and blood lactic acid. Throughout this experiment there seems to be a constant tendency of simultaneous divergence or convergence of the curves.

Correcting for the gradient of the curve of elimination of non-oxidative carbon dioxide the graph shows a very small carbon dioxide index. Following the first injection, the carbon dioxide index for the inclusive period from injection to gas sample 13 is 0.18 and for the second injection up to gas sample 16 the index is 0.25.

The curve of energy production shows very little change during the first injection and recovery but for the second injection the energy liberated by the rapid formation of lactic acid more than compensates for the loss due to oxidations and the curve shows a sharp rise. The energy production at gas samples 14 and 15 is higher than the initial value despite the very low rate of oxidations. Consulting the table of oxygen lactic acid ratios and carbon dioxide indices it will be seen that the amount of lactic acid formed per unit of shortage of oxygen is large. The ratios of oxygen shortage over lactic acid increase are small; for the first period of injection and recovery it is 23 and for the second period of injection and recovery it is 75. For the first two periods during the more rapid formation of lactic acid it is 27 and 53 respectively.

*Experiment 5.* There were five injections of sodium cyanide and one injection of isotonic saline solution. The last three injections of sodium cyanide occurring at 77 minutes 21 seconds, 83 minutes 48 seconds, and 90 minutes 48 seconds occur in such rapid succession that they may be first considered as one injection. The general effect of all of the injections of cyanide was a drop in the gaseous metabolism. Oxygen absorption fell from 397.7 cc. per kgm. hour to 203.4 cc. Carbon dioxide elimination dropped from 345.3 cc. per kgm. hour to 197.3 cc. The expiratory quotient increased from 0.87 to 0.97. Blood lactic acid increased from 37 mgm. per 100 cc. of blood to 98.5 grams. The carbon dioxide capacity of the blood decreased from 59.20 cc. to 44.50 cc. and the pH of the blood decreased from 7.50 to 7.30. The immediate effects of injections were superimposed on these general effects. Each of the three injections produced an appreciable accumulation of lactic acid in the blood. During recovery after the first injection there was a fairly rapid disappearance of lactic

TABLE 5

*Experiment 5*

| OBSERVATION | PROCEDURE | TIME | PER CENT OF EXPIRED $CO_2$ | PER CENT OF EXPIRED $O_2$ | EXPIRATORY QUOTIENT | $O_2$ CONSUMPTION IN CC. PER KGM. HR. | $CO_2$ ELIMINATION IN CC. PER KGM. HR. | $CO_2$ ELIMINATION FROM OXIDATIONS BASED ON E.Q. IN CC. PER KGM. HR. | DIFFERENCE BETWEEN ACTUAL AND THEORETICAL $CO_2$ ELIMINATION | $CO_2$ EQUIVALENT OF CHANGE OF LACTIC ACID | LACTIC ACID IN MGM. PER 100 CC. ARTERIAL BLOOD | $CO_2$ CAPACITY OF ARTERIAL BLOOD IN VOLUME PER CENT | pH OF ARTERIAL BLOOD | MEAN BLOOD PRESSURE | OXIDATIVE ENERGY IN SMALL CALORIES PER KGM. HR. | NON-OXIDATIVE ENERGY IN SMALL CALORIES PER KGM. HR. | OXIDATIVE + NON-OXIDATIVE ENERGY IN SMALL CALORIES PER KGM. HR. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 |
| 1 | G. S. no. 1 | 9′ 28″ | 3.42 | 17.36 | 0.93 | 389 | 362.2 | | | | | | | 80 | 1,874 | | |
| 2 | A. S. no. 1 | 9′ 38″ | | | | | | | | | 38.7 | 58.8 | 7.50 | | | | |
| 3 | G. S. no. 2 | 21′ 10″ | 3.27 | 17.32 | 0.87 | 397.7 | 345.3 | | | | | | | 77 | 1,915 | −21 | |
| 4 | A. S. no. 2 | 21′ 11″ | | | | | | | | | 37.4 | 59.20 | 7.51 | | | | |
| 5 | 50 cc. 0.0033 n NaCN | 24′ 45″ | | | | | | | | | c 37.0 | | | 66 | | | |
| 6 | G. S. no. 3 | 30′ 41″ | 3.29 | 17.45 | 0.92 | 378.1 | 346.2 | 328.2 | +18 | 454 | | | | 74 | 1,821 | +551 | 2,372 |
| 7 | A. S. no. 3 | 31′ 16″ | | | | | | | | | 56.7 | 58.50 | 7.44 | | | | |
| 8 | G. S. no. 4 | 34′ 5″ | 3.56 | 17.28 | 0.95 | 394.6 | 376.2 | 342.6 | +34 | 167 | | | | 65 | 1,900 | +203 | 1,697 |
| 9 | G. S. no. 5 | 38′ 44″ | 3.30 | 17.47 | 0.92 | 376.7 | 347.4 | 327 | +20 | 167 | | | | 62 | 1,815 | +203 | 1,612 |
| 10 | A. S. no. 4 | 38′ 44″ | | | | | | | | | 48.4 | 56.33 | 7.49 | | | | |
| 11 | G. S. no. 6 | 48′ 56″ | 3.16 | 17.33 | 0.83 | 398.6 | 332.5 | 346 | −14 | +8 | | | | 48 | 1,920 | +11 | 1,931 |
| 12 | A. S. no. 5 | 48′ 56″ | | | | | | | | | 49.0 | 54.80 | 7.50 | | | | |
| 13 | 50 cc. 0.0033 NaCN | 51′ 22″ | | | | | | | | | c 49.1 | | | 48 | | | |
| 14 | G. S. no. 7 | 58′ 22″ | 2.99 | 17.95 | 0.98 | 321.5 | 315.6 | 279 | +37 | +104 | | | | 56 | 1,548 | +136 | 1,684 |
| 15 | A. S. no. 6 | 58′ 26″ | | | | | | | | | 54.0 | 53.40 | 7.45 | | | | |
| 16 | G. S. no. 8 | 61′ 57″ | 3.33 | 17.57 | 0.97 | 370.0 | 359.1 | 321 | +38 | +112 | | | | 64 | 1,782 | +136 | 1,918 |
| 17 | G. S. no. 9 | 67′ 25″ | 3.18 | 17.51 | 0.90 | 382.1 | 341.8 | 331.7 | +10 | +112 | | | | 54 | 1,835 | +136 | 1,971 |

| | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 18 | A. S. no. 7 | 67′ 40″ | | | | | | | | | 60.9 | 50.31 | 7.45 | | | | |
| 19 | G. S. no. 10 | 74′ 21″ | 2.85 | 18.03 | 0.95 | 321.3 | 306.6 | 279 | +28 | +20 | | | | 46 | 1,548 | +25 | 1,573 |
| 20 | A. S. no. 8 | 74′ 21″ | | | | | | | | | 61.8 | 51.01 | 7.45 | | | | |
| 21 | 50 cc. 0.005 n NaCN | 77′ 21″ | | | | | | | | | c 62.1 | | | 40 | | | |
| 22 | 50 cc. 0.005 n NaCN | 83′ 48″ | | | | | | | | | | | | | | | |
| 23 | G. S. no. 11 | 85′ 42″ | 2.80 | 18.41 | 1.12 | 275.3 | 307.7 | 239 | +69 | +212 | | | | 50 | 1,326 | +258 | 1,584 |
| 24 | G. S. no. 12 | 88′ 23″ | 2.98 | 18.18 | 1.09 | 301.9 | 327.5 | 262 | +66 | +212 | | | | 56 | 1,454 | +258 | 1,712 |
| 25 | A. S. no. 9 | 88′ 23″ | | | | | | | | | 77.7 | 49.48 | 7.33 | | | | |
| 26 | 50 cc. 0.005 m NaCN | 90′ 48″ | | | | | | | | | | | | | | | |
| 28 | G. S. no. 13 | 98′ | 2.62 | 18.44 | 1.04 | 275.9 | 287 | 239.6 | 47 | +264 | | | | 42 | 1,328 | +322 | 1,650 |
| 29 | A. S. no. 10 | 98′ | | | | | | | | | 90.4 | 48.02 | 7.39 | | | | |
| 30 | R. ② | 104′ | | | | | | | | | | | | | | | |
| 31 | G. S. no. 14 | 109′ 55″ | 1.83 | 19.11 | 0.97 | 203.4 | 197.3 | 176.5 | +21 | +92 | | | | 25 | 980 | +112 | 1,102 |
| 32 | A. S. no. 11 | 109′ 55″ | | | | | | | | | 97.7 | 44.54 | 7.30 | | | | |
| 33 | 1000 cc. saline | 111′ 13″ | | | | | | | | | c 98.5 | | | | | | |
| 34 | G. S. no. 15 | 114′ 2″ | 4.03 | 16.97 | 1.01 | 437.4 | 440.2 | 380 | +60 | −230 | | | | 66 | 2,108 | −280 | 1,828 |
| 35 | A. S. no. 11A | 115′ 40″ | | | | | | | | | | | 7.41 | | | | |
| 36 | G. S. no. 16 | 116′ 25″ | 4.32 | 17.01 | 1.12 | 425.4 | 474.2 | 369.3 | +105 | −230 | | | | 76 | 2,050 | −280 | 1,770 |
| 37 | G. S. no. 17 | 122′ 17″ | 3.22 | 17.57 | 0.93 | 378.8 | 351.7 | 328.8 | +23 | −230 | | | | 64 | 1,824 | −280 | 1,544 |
| 37A | A. S. no. 12 | 122′ 17″ | | | | | | | | | 81.4 | 45.90 | 7.41 | | | | |
| 38 | G. S. no. 18 | 131′ 53″ | 2.41 | 18.16 | 0.82 | 318.8 | 261.4 | 276.8 | −16 | −112 | | | | 48 | 1,534 | −137 | 1,397 |
| 39 | A. S. no. 13 | 131′ 53″ | | | | | | | | | 74.2 | 48.63 | 7.44 | | | | |

acid. During recovery following the last two injections there was no disappearance of lactic acid from the blood. Only the rate of the preceding accumulation was reduced. Each accumulation of lactic acid was accompanied by a rise in the expiratory quotient and each period of recovery by a fall. Though the total amount of lactic acid accumulated in the blood is relatively large the amount of non-oxidative carbon dioxide eliminated is relatively small. For the total period from the first injection of cyanide to gas sample 14 the carbon dioxide index is 0.26. While the

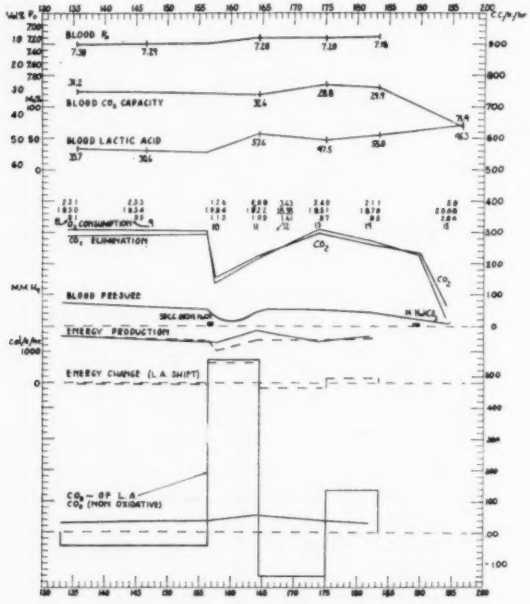



Fig. 6. Experiment 6

general tendency of energy production is downward there are three high points in the curve where the rate of lactic acid accumulation with respect to the shortage of oxygen consumption is relatively high. At gas sample 3 the total energy increased from a normal value of 1915 calories per kgm. hour to 2372 calories. The corresponding ratio of oxygen shortage to lactic acid increase was 3. At gas sample 9 the energy production was again above normal—1971 calories. The corresponding oxygen lactic acid ratio was 51. At gas sample 12 the energy production increased again but not above normal. The corresponding oxygen-lactic acid ratio was 72. The oxygen lactic acid ratio corresponding to the general drop in

TABLE 6

*Experiment 6*

| OBSERVATION | PROCEDURE | TIME | PER CENT OF EXPIRED $CO_2$ | PER CENT OF EXPIRED $O_2$ | RESPIRATORY QUOTIENT | $O_2$ CONSUMPTION IN CC. PER KGM. HR. | $CO_2$ ELIMINATION IN CC. PER KGM. HR. | $CO_2$ ELIMINATION FROM OXIDATIONS BASED ON E.Q. IN CC. PER KGM. HR. | DIFFERENCE BETWEEN ACTUAL AND THEORETICAL $CO_2$ ELIMINATION | $CO_2$ EQUIVALENT OF CHANGE OF LACTIC ACID | LACTIC ACID IN MGM. PER 100 CC. ARTERIAL BLOOD | LACTIC ACID IN MGM. PER 100 CC. VENOUS BLOOD | $CO_2$ CAPACITY OF ARTERIAL BLOOD IN VOLUME PER CENT | $CO_2$ CAPACITY OF VENOUS BLOOD IN VOLUME PER CENT | pH OF ARTERIAL BLOOD | pH OF VENOUS BLOOD | MEAN BLOOD PRESSURE | OXIDATIVE ENERGY IN SMALL CALORIES PER KGM. HR. | NON-OXIDATIVE ENERGY IN SMALL CALORIES PER KGM. HR. | OXIDATIVE + NON-OXIDATIVE ENERGY IN SMALL CALORIES PER KGM. HR. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12A | 12V | 13A | 13V | 14A | 14V | 15 | 16 | 17 | 18 |
| 22 | G. S. no. 8 | 133′ 8″ | 2.31 | 18.50 | 0.91 | 310 | 288.1 | 258.5 | +30 | −14 | | | | | | | 74 | 1,500 | −16 | 1,484 |
| 23 | V. S. no. 6 | 134′ 42″ | | | | | | | | | | 36.2 | | 38.89 | | 7.24 | | | | |
| 24 | A. S. no. 6 | 135′ 40″ | | | | | | | | | 33.7 | | 31.15 | | 7.30 | | | | | |
| 25 | G. S. no. 9 | 144′ 41″ | 2.33 | 18.54 | 0.95 | 307.6 | 290.6 | 256.5 | +34 | −43 | | | | | | | 64 | 1,440 | −51 | 1,389 |
| 26 | V. S. no. 7 | 145′ 38″ | | | | | | | | | | 39.7 | | 36.78 | | 7.24 | | | | |
| 27 | A. S. no. 7 | 146′ 30″ | | | | | | | | | 30.6 | | | | 7.29 | | | | | |
| 28 | 0.0033 n NaCN | 156′ 20″ | | | | c 306 | | | | | c 28 | c 43 | | | | | 54 | | | |
| 29 | G. S. no. 10 | 157′ 45″ | 1.26 | 19.84 | 1.13 | 136.7 | 155.0 | 114 | +41 | +544 | | | | | | | 20 | 660 | +620 | 1,280 |
| 30 | G. S. no. 11 | 164′ 5″ | 1.88 | 19.22 | 1.09 | 215.2 | 234.2 | 179.5 | +55 | +544 | | | | | | | 45 | 1,040 | +620 | 1,680 |
| 31 | V. S. no. 8 | 163′ 38″ | | | | | | | | | | 43.4 | | 34.8 | | 7.23 | | | | |
| 32 | A. S. no. 8 | 164′ 29″ | | | | | | | | | 57.6 | | 32.6 | | 7.20 | | | | | |
| 33 | G. S. no. 12 | 167′ 15″ | 3.63 | 18.38 | 1.61 | 288 | 463 | | | | | | | | | | 52 | | | |
| 34 | G. S. no. 13 | 173′ 53″ | 2.40 | 18.51 | 0.97 | 310.5 | 299.7 | 258.5 | +41 | −143 | | | | | | | 52 | 1,500 | +68 | 1,332 |
| 35 | V. S. no. 9 | 174′ 38″ | | | | | | | | | | | | 28.28 | | 7.16 | | | | |
| 36 | A. S. no. 9 | 175′ 6″ | | | | | | | | | 47.5 | | 28.81 | | 7.20 | | | | | |
| 37 | G. S. no. 14 | 182′ 2″ | 2.11 | 18.79 | 0.95 | 275.5 | 262.0 | 229.5 | +32 | +135 | | | | | | | 44 | 1,330 | +162 | 1,492 |
| 38 | V. S. no. 10 | 182′ 52″ | | | | | | | | | | | | 33.9 | | 7.15 | | | | |
| 39 | A. S. no. 10 | 183′ 28″ | | | | | | | | | 55.0 | | 29.90 | | 7.18 | | | | | |
| 39A | $NaHCO_3$ | 189′ | | | | | | | | | | | | | | | | | | |
| 40 | G. S. no. 15 | 194′ 28″ | 0.58 | 20.68 | 2.84 | 24.2 | 68.83 | 20 | +49 | +186 | | | | 38.74 | | | 6 | 120 | +225 | 345 |
| 41 | V. S. no. 11 | 196′ 6″ | | | | | | | | | | 80.4 | 45.31 | | | | | | | |
| 42 | A. S. no. 11 | 196′ 40″ | | | | | | | | | 71.4 | | | | | | | | | |
| | Stopped drum | 206′ | | | | | | | | | | | | | | | | | | |

TABLE 7

*Summary of carbon dioxide indices with oxygen-lactic acid ratios*

| PERIOD | VARIATIONS IN $O_2$ CONSUMPTION CMM./KGM. (+ = EXCESS; − = SHORTAGE) | VARIATIONS IN LACTIC ACID MGM./KGM. (+ = INCREASE; − = DECREASE) | $O_2$ SHORTAGE CMM./KGM. / LACTIC ACID INCREASE MGM./KGM. | NON-OXIDATIVE $CO_2$ CC./KGM. (+ = ELIMINATION; − = RETENTION) | $CO_2$ — LACTIC ACID CHANGES (CC./KGM. (+ = INCREASE IN LACTIC ACID; − = DECREASE) | + NON-OXIDATIVE $CO_2$ / $CO_2$ — LACTIC ACID INCREASE |
|---|---|---|---|---|---|---|
| **Experiment 1** | | | | | | |
| NaCN to G. S. 11 | −10,030 | +205 | 49 | +4.35 | +51.25 | 0.08 |
| G. S. 11 to G. S. 12 | −4,330 | +70 | 62 | +2.15 | +17.50 | 0.12 |
| G. S. 12 to G. S. 13 | −1,180 | +45 | 26 | +2.09 | +11.25 | 0.19 |
| NaCN to G. S. 15 | −17,750 | +280 | 64 | +16.65 | +70.00 | 0.24 |
| $NaHCO_3$ to G. S. 18 | +43,940 | +210 | | +49.60 | +52.50 | 0.95 |
| **Experiment 2** | | | | | | |
| NaCN to G. S. 3 | −5,660 | +140 | 40 | +5.16 | +35.00 | 0.15 |
| G. S. 3 to G. S. 4 | −20,910 | +150 | 139 | +12.08 | +37.50 | 0.32 |
| G. S. 4 to G. S. 5 | −3,520 | +25 | 141 | +1.47 | +6.00 | 0.25 |
| G. S. 5 to G. S. 6 | −9,890 | +35 | 282 | +5.63 | +8.75 | 0.65 |
| G. S. 6 to G. S. 7 | −6,720 | +40 | 168 | +4.30 | +10.00 | 0.43 |
| G. S. 7 to G. S. 8 | −36,580 | −10 | | +18.77 | −2.50 | |
| G. S. 8 to G. S. 9 | −59,280 | +33 | 1797 | +23.36 | +8.25 | 2.81 |
| NaCN to G. S. 4 | −26,570 | +290 | 95 | +17.24 | +72.50 | 0.24 |
| NaCN to G. S. 8 | −83,280 | +379 | 220 | +47.41 | +94.75 | 0.50 |
| NaCN to G. S. 9 | −142,560 | +412 | 341 | +70.77 | +103.00 | 0.68 |

Experiment 3

| | | | | | | |
|---|---|---|---|---|---|---|
| NaCN to G. S. 21 | −17,350 | +300 | 58 | +30.36 | +75.00 | 0.40 |
| NaCN to G. S. 23 | −27,960 | +220 | 128 | +47.51 | +55.00 | 0.86 |
| NaCN to G. S. 21* | | | | +16.53 | 75.00 | 0.22 |
| NaCN to G. S. 23* | | | | +28.94 | 55.00 | 0.52 |

Experiment 4

| | | | | | | |
|---|---|---|---|---|---|---|
| 1st NaCN to G. S. 11 | −2,410 | 65 | 27 | +7.72 | +16.25 | 0.48 |
| 1st NaCN to G. S. 13* | +850 | +80 | | +17.45 | +20.00 | 0.87 |
| 2nd NaCN to G. S. 14 | −1,160 | 50 | 23 | +1.03 | +12.50 | 0.08 |
| G. S. 14 to G. S. 15 | −11,530 | +220 | 53 | +9.19 | +55.00 | 0.18 |
| G. S. 15 to G. S. 16 | −17,230 | +130 | 133 | +15.13 | +32.50 | 0.47 |
| 2nd NaCN to G. S. 16 | −29,920 | +400 | 75 | +25.35 | +100.00 | 0.25 |

Experiment 5

| | | | | | | |
|---|---|---|---|---|---|---|
| NaCN to G. S. 3 | −660 | +180 | 3 | +.88 | +45.00 | 0.02 |
| G. S. 8 to G. S. 9 | −2,160 | +42 | 51 | +2.20 | +10.50 | 0.21 |
| G. S. 11 to G. S. 12 | −5,040 | +70 | 72 | +5.50 | +17.50 | 0.31 |
| 1st NaCN to G. S. 14 | −94,970 | +610 | 156 | +39.77 | +152.50 | 0.26 |
| 1st NaCN to G. S. 13* | | | | +3.56 | +20.00 | 0.18 |
| 2nd NaCN to G. S. 16* | | | | +22.08 | +100.00 | 0.22 |

Experiment 6

| | | | | | | |
|---|---|---|---|---|---|---|
| NaCN to G. S. 10 | −1,780 | +50 | 36 | +.83 | +12.50 | 0.07 |
| G. S. 10 to G. S. 11 | −12,890 | +225 | 57 | +3.68 | +56.25 | 0.07 |
| NaCN to G. S. 12 | −21,530 | +205 | 106 | +11.50 | +51.25 | 0.29 |
| NaCN to G. S. 13 | −22,970 | +255 | 90 | +16.29 | +63.75 | 0.26 |

* Using gradient.

energy production from the first injection of cyanide to gas sample 14 was 156 which is about twice the ratio which maintains energy production at a constant level.

The injection of 1000 cc. saline solution increased gaseous metabolism enormously. There was a corresponding decrease in blood lactic acid and hydrogen ion concentration and an increase in the carbon dioxide capacity of the blood. Attention is called to two points of interest. The hydrogen ion concentration diminished despite the increase in carbon dioxide of the expired air from 1.83 per cent to 4.32 per cent, and the expiratory quotient increased from 0.97 to 1.12 despite the rapid disappearance of lactic acid. The decrease in hydrogen ion concentration of the blood is probably accounted for by the decrease in blood lactic acid and the increase in the expiratory quotient by the improved ventilation of the blood and tissues resulting from the increased flow of blood. That the ventilation of the blood and tissues is improved is indicated by the large increase in elimination of non-oxidative carbon dioxide, and assuming a partial non-oxidative removal of lactic acid the effects of increased flow of blood are greater than the expiratory quotients indicate. The total energy production is markedly increased even assuming that all of the lactic acid was removed by non-oxidative processes.

*Experiment 6.* The graph shows effects of a very rapid injection of sodium cyanide towards the close of the experiment—at 156 minutes 20 seconds. The previous history is—administration of 5.21 per cent oxygen mixture at 47 minutes 15 seconds, readministration of room air at 53 minutes 56 seconds, and intravenous injection of saline solution at 93 minutes. This experiment is one of the few in which cyanide was rapidly injected. Oxygen absorption and carbon dioxide elimination fell off with corresponding rapidity. The marked fall in blood pressure indicates that much of the reduction of the gaseous metabolism is to be accounted for by the reduction in the total volume flow of blood. This is supported by the record of volume flow through the femoral artery. The decreased oxygen absorption and increased lactic acid are associated with the usual increase in the expiratory quotient but the decrease in carbon dioxide capacity of the blood is missing. The curves of blood carbon dioxide capacity and blood lactic acid approach each other as in some of the preceding experiments. The hydrogen ion concentration of the blood increased as it did in all of the preceding experiments. With return of oxidations there was a temporary decrease in blood lactic acid but again the carbon dioxide capacity of the blood changed in the unexpected direction and the curves of blood carbon dioxide capacity and blood lactic acid diverge. As oxidations fall again the curves once more converge. The final rapid convergence is due to an injection of sodium bicarbonate administered to support the failing circulation. The theoretical maximum non-oxidative

carbon dioxide liberated by the lactic acid with respect to the actual non-oxidative carbon dioxide liberated is very large and the elimination of non-oxidative carbon dioxide very small. For the period intervening between injection of sodium cyanide and gas sample 12 the carbon dioxide index is 0.29, and for the more inclusive period up to gas sample 13 it is 0.26. For the limited period of increasing lactic acid from the administration of sodium cyanide to gas sample 11 the index is very much lower 0.07. The low ratio of oxygen shortage to lactic acid increase during the early periods of impaired oxidation, 0.36 and 0.57, leads to a production of energy considerably in excess of the normal value. The energy production increased from 1389 calories per hour to a maximum of 1680.

## SUMMARY

The effects of intravenous injection of sodium cyanide on numerous factors relating to respiratory control are studied with the methods described in the preceding paper.

With room air continuously administered at a constant volume intravenous injection of sodium cyanide commonly decreased oxygen consumption and carbon dioxide elimination. On recovery oxygen consumption and carbon dioxide elimination increased. The hyper oxygen consumption and hyper carbon dioxide elimination common to recovery on readministration of room air following anoxemia was seldom obtained. This perhaps was due to the permanent injury of the oxidative mechanism of the tissues. The tendency toward overshooting during incomplete recovery, however, was quite apparent.

As in the anoxemia experiments the decrease in carbon dioxide elimination was less than the increase in oxygen consumption. Injection of sodium cyanide was, therefore, accompanied by an increase in the expiratory quotient and recovery was accompanied by a decrease. Contrary to the anoxemia experiments the expiratory quotient of recovery never fell below normal levels.

Intravenous injection of sodium cyanide was associated with a rapid increase in the lactic acid content of the blood. Recovery was associated with a decrease or with a less rapid increase. Lactic acid levels at the beginning and end of experiments indicated incomplete recovery of the oxidative mechanism.

In contrast to the experiments with lowered alveolar oxygen the immediate effects of intravenous injection were increased hydrogen ion concentration of the arterial blood. The absence of an alkaline change is presumably attributable to an absence of reduction of hemoglobin.

Assuming equal distribution of lactic acid between blood and tissues the proportion of lactic acid reacting with bicarbonate as indicated by the

elimination of non-oxidative carbon dioxide was considerably smaller than in the low oxygen experiments.

Assuming equal distribution of lactic acid between blood and tissues the rate of lactic acid production per unit of oxygen shortage was commonly higher than in the low oxygen experiments. Total energy production (oxidative plus non-oxidative) was frequently greater than normal during periods of impaired oxidations.

## BIBLIOGRAPHY

HENRIQUES. 1929. Ergebn. d. Physiol., xxviii, 625.
GESELL AND HERTZMAN. 1928. This Journal, lxxxiii, 420.
McGINTY AND GESELL. 1927. This Journal, lxxxiii, 345.

# THE REGULATION OF RESPIRATION

## A Study of the Correlation of Numerous Factors of Respiratory Control During Hemorrhage and Reinjection[1,2]

ROBERT GESELL, HUGO KRUEGER,[3] GRACE GORHAM AND THEODORE BERNTHAL

*From the Department of Physiology, University of Michigan, Ann Arbor*

Received for publication March 17, 1930

In the two preceding series of experiments tissue oxidations were impaired by two distinctly different methods—reduction in the supply of oxygen to the tissues by lowering the alveolar oxygen pressure and impairment of the oxidative mechanism by the intravenous injection of sodium cyanide. In the present experiments hemorrhage and reinjection are employed to vary oxidations by modifying the volume flow of blood. While the supply of oxygen to the tissues is reduced by hemorrhage and by lowered alveolar oxygen the mechanisms involved are somewhat different. With lowered alveolar oxygen the arterial blood is poorly oxygenated and the total flow most probably augmented. In hemorrhage the arterial blood is hyperoxygenated and the flow is less than normal. Since in hemorrhage the venous blood is darker than normal we must picture a highly oxygenated blood undergoing a large reduction in oxygen content and oxygen pressure as it flows at a retarded rate through the tissues. At the venous end of the capillaries the tissues must, therefore, suffer from a lowered oxygen pressure as they do with lowered alveolar oxygen pressure. Just as the unloading of oxygen per unit flow of blood is increased by hemorrhage so is the loading of the blood with acid increased per unit flow through the tissues. This was clearly indicated by simultaneous determination of the hydrogen ion concentration of arterial and venous blood during temporary acute hemorrhage (Hertzman and Gesell, 1927). In such experiments the arterial blood turned alkaline due to augmented pulmonary ventilation and the retarded flow of blood through the lungs, nevertheless the venous blood turned more acid than normal due to the retarded flow

[1] Preliminary report, This Journal, 1929, xc, 359.

[2] This investigation has been made with the assistance of a grant from the Committee on Therapeutic Research, Council on Pharmacy and Chemistry, American Medical Association.

[3] Submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

of blood through the tissues. The difference in the hydrogen ion concentration of the arterial and venous blood had, therefore, been increased, indicating that a mechanism is at work tending to maintain a relatively constant acid base equilibrium of the tissues despite the reduction in volume flow of blood. It is thus conceivable that if hemorrhage fails to reduce the total oxidations, the acid base equilibrium of the body as a whole is little affected. The greater reduction of oxyhemoglobin which is entailed by the slower flow of blood through the tissues would lead to a greater liberation of base to accommodate the heavier load of carbon

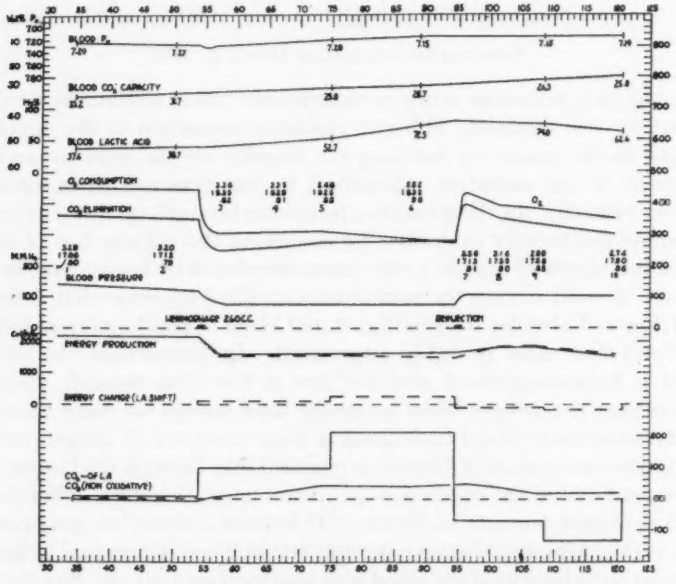



Fig. 1. Experiment 1

dioxide. There would consequently be no broken coördination of the dual function of hemoglobin. On the other hand if oxidations are impaired and lactic acid formation increased, a broken coördination of the dual function of hemoglogin is established just as it is with lowered alveolar oxygen and cyanide poisoning. Thus in addition to the impairment of the physical transport of carbon dioxide (resulting from a decrease in flow of blood as a physical solvent) a chemical change is established leading to altered acid metabolism and altered acid accumulation in the tissues. Hemorrhage thus presents problems which are worthy of more detailed study.

METHOD. The effects of hemorrhage and reinjection of blood were studied by the methods of the preceding paper. Room air was continually administered at a constant volume and changes in blood volume produced by hemorrhage and reinjection through the femoral artery and vein respectively. Blood and gas samples were taken as before.

RESULTS. *Experiment 1.* At the beginning of the experiment at 0 minute 300 cc. of saline solution were injected. Gas samples 1 and 2 taken at 31 minutes 36 seconds and 48 minutes and 18 seconds indicate that oxygen absorption was high and constant—451.8 cc. and 445 cc. per kgm. hour respectively. The expiratory quotients were 0.80 and 0.79. The blood changes during this time were almost as small as those of gaseous metabolism. The hydrogen ion concentration decreased from a pH of 7.24 to 7.27, the blood carbon dioxide capacity decreased slightly and blood lactic acid was virtually unchanged. At 54 minutes 5 seconds—260 cc. of blood were drawn but since the animal began to fail too rapidly 25 cc. of blood were returned at once. The immediate result of this hemorrhage was a sudden drop in oxygen absorption and carbon dioxide elimination of nearly equal proportions so that at gas samples 3 and 4 the expiratory quotients were only slightly elevated to 0.82 and 0.81 respectively. From gas samples 3 to 4 to 5 oxygen absorption was nearly constant. The slight change was in the upward direction from 303.9 to 306.9 to 309.6 cc. per kgm. hour respectively. Carbon dioxide elimination increased at a slightly faster rate so that at gas sample 5 the expiratory quotient had risen to 0.88. From gas sample 5 to 6 and to the end of hemorrhage the initial slight improvement in gaseous metabolism gave way to a relatively rapid failure. Oxygen absorption diminished and carbon dioxide elimination increased with a resulting increase in the expiratory quotient to 0.98 at gas sample 6. Before injection the oxygen and carbon dioxide curves had crossed, meaning that the expiratory quotient had risen above unity. Regarding the explanation of the increased expiratory quotient during hemorrhage we recall our suggestion that the increased expiratory quotient of anoxemia and cyanide poisoning was due not only to a liberation of preformed carbon dioxide by the formation of lactic acid but in part by increased volume flow of blood. Clearly changes in volume flow of blood cannot be employed to explain the increased expiratory quotient of hemorrhage where volume flow is definitely decreased. We have found, as did Riegel (1927), that hemorrhage leads to accumulation of lactic acid in the blood. The increased expiratory quotient is, therefore, explained primarily on chemical grounds. It is quite evident that where the increase in blood lactic acid is slow the rise in the expiratory quotient is relatively small and toward the end of hemorrhage where the increase in lactic acid is more rapid the increase in the expiratory quotient is correspondingly rapid.

TABLE 1

*Experiment 1. Hemorrhage*

| OBSERVATION | PROCEDURE | TIME | PER CENT OF EXPIRED $CO_2$ | PER CENT OF EXPIRED $O_2$ | EXPIRATORY QUOTIENT | $O_2$ CONSUMPTION IN CC. PER KGM. HR. | $CO_2$ ELIMINATION IN CC. PER KGM. HR. | $CO_2$ ELIMINATION FROM OXIDATIONS BASED ON E.Q. IN CC. PER KGM. HR. | DIFFERENCE BETWEEN ACTUAL AND THEORETICAL $CO_2$ ELIMINATION | $CO_2$ EQUIVALENT OF CHANGE OF LACTIC ACID | LACTIC ACID IN MGM. PER 100 CC. ARTERIAL BLOOD | LACTIC ACID IN MGM. PER 100 CC. VENOUS BLOOD | $CO_2$ CAPACITY OF ARTERIAL BLOOD IN VOL. PER CENT | $CO_2$ CAPACITY OF VENOUS BLOOD IN VOL. PER CENT | pH OF ARTERIAL BLOOD | pH OF VENOUS BLOOD | MEAN BLOOD PRESSURE | OXIDATIVE ENERGY IN SMALL CALORIES PER KGM. HR. | NON-OXIDATIVE ENERGY IN SMALL CALORIES PER KGM. HR. | OXIDATIVE + NON-OXIDATIVE ENERGY IN SMALL CALORIES PER KGM. HR. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12A | 12V | 13A | 13V | 14A | 14V | 15 | 16 | 17 | 18 |
| 0 | Saline 300 cc. | 0 | | | | | | | | | | | | | | | | | | |
| 1 | G. S. no. 1 | 31′ 36″ | 3.29 | 17.06 | 0.80 | 451.8 | 362.1 | 362 | | | | | | | | | 140 | 2,172 | | |
| 2 | V. S. no. 1 | 33′ 8″ | | | | | | | | | | 38.3 | | 32.37 | | 7.27 | | | | |
| 3 | A. S. no. 1 | 34′ 19″ | | | | | | | | | 37.6 | | 33.17 | | 7.24 | | | | | |
| 4 | G. S. no. 2 | 48′ 18″ | 3.20 | 17.12 | 0.79 | 445.5 | 352.3 | 357 | −5 | | | | | | | | 125 | 2,144 | | |
| 5 | V. S. no. 2 | 49′ 10″ | | | | | | | | | | 39.5 | | 32.20 | | 7.29 | | | | |
| 6 | A. S. no. 2 | 50′ 5″ | | | | | | | | | 38.7 | | 31.69 | | 7.27 | | | | | |
| 7 | Hem. 260 cc. | 54′ 5″ | | | | | | | | | c 39.0 | c 40.2 | | | | | 117 | | | |
| 8 | G. S. no. 3 | 57′ 20″ | 2.26 | 18.33 | 0.82 | 303.9 | 249.1 | 243.8 | 6 | 98 | | | | | | | | 1,464 | +119 | 1,583 |
| 9 | Inj. 25 cc. blood | 58′ 27″ | | | | | | | | | | | | | | | 75 | | | |
| 10 | G. S. no. 4 | 66′ 1″ | 2.35 | 18.29 | 0.81 | 306.9 | 258.1 | 246 | 12 | 98 | | | | | | | 76 | 1,477 | +119 | 1,596 |
| 11 | G. S. no. 5 | 73′ 32″ | 2.48 | 18.25 | 0.88 | 309.6 | 273.6 | 248.2 | 25 | 98 | | | | | | | | 1,491 | +119 | 1,610 |
| 12 | V. S. no. 3 | 74′ 22″ | | | | | | | | | | 55.1 | | 24.91 | | 7.14 | | | | |
| 13 | A. S. no. 3 | 74′ 57″ | | | | | | | | | 52.7 | | 24.97 | | 7.20 | | 75 | | | |
| 14 | G. S. no. 6 | 87′ 22″ | 2.52 | 18.32 | 0.98 | 284 | 278.4 | 237.8 | 41 | 214 | | | | | | | | 1,367 | +260 | 1,627 |
| 15 | V. S. no. 4 | 88′ 9″ | | | | | | | | | | 73.4 | | 24.96 | | 7.11 | | | | |
| 16 | A. S. no. 4 | 88′ 49″ | | | | | | | | | 72.5 | | 28.69 | | 7.15 | | 75 | | | |

| | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 17 | Reinjection | 94′ 30″ | | | | | | | | | c 80.3 | c 82.3 | | | | | 75 | | | | |
| | 235 cc. | | | | | | | | | | | | | | | | | 2,094 | −84 | 2,010 | |
| 18 | G. S. no. 7 | 96′ 17″ | 3.58 | 17.13 | 0.91 | 435 | 396.5 | 348.8 | 48 | −68 | | | | | | | 106 | | | | |
| 19 | | 97′ 52″ | | | | | | | | | | | | | | | | 1,891 | −84 | 1,807 | |
| 20 | G. S. no. 8 | 101′ 15″ | 3.16 | 17.55 | 0.90 | 392.9 | 353.3 | 315 | 38 | −68 | | | | | | | 114 | 1,890 | −84 | 1,806 | |
| 21 | G. S. no. 9 | 107′ 9″ | 2.99 | 17.58 | 0.85 | 392.3 | 333.5 | 314.5 | 19 | −68 | | 76.8 | | 25.12 | | 7.10 | | | | | |
| 22 | V. S. no. 5 | 107′ 50″ | | | | | | | | | 74.0 | | 26.26 | | 7.15 | | | | | | |
| 23 | A. S. no. 5 | 108′ 30″ | | | | | | | | | | | | | | | 100 | 1,683 | −161 | 1,521 | |
| 24 | G. S. no. 10 | 119′ 39″ | 2.74 | 17.90 | 0.86 | 349.4 | 301.0 | 280.2 | 21 | −132 | | 71.2 | | 24.75 | | 7.10 | | | | | |
| 25 | V. S. no. 6 | 120′ 10″ | | | | | | | | | 62.6 | | 24.98 | | 7.14 | | | | | | |
| 26 | A. S. no. 6 | 120′ 45″ | | | | | | | | | | | | | | | | | | | |
| | Stopped drum. Changed paper | | | | | | | | | | | | | | | | 88 | 1,524 | +124 | 1,648 | |
| 27 | G. S. no. 11 | 141′ 37″ | 2.48 | 18.17 | 0 85 | 316.7 | 270.2 | 254 | 16 | +102 | | 82.6 | | 20.51 | | 7.08 | | | | | |
| 28 | V. S. no. 7 | 142′ 38″ | | | | | | | | | 78.1 | | 22.61 | | 7.15 | | | | | | |
| 29 | A. S. no. 7 | 143′ 37″ | | | | | | | | | c 83.5 | c 87.5 | | | | | 78 | | | | |
| 30 | m/$NaHCO_3$, 200 cc. | 152′ 1″ | | | | | | | | | | | | | | | | 1,920 | +626 | 2,546 | |
| 31 | G. S. no. 12 | 154′ 45″ | 5.97 | 16.94 | 1.70 | 399 | 677.8 | 320 | 358 | +515 | | | | | | | 95 | 2,202 | +626 | 2,828 | |
| 32 | G. S. no. 13 | 155′ 29″ | 5.98 | 16.52 | 1.48 | 457.2 | 675.3 | 368 | 307 | +515 | | | | | | | 105 | 2,090 | +626 | 2,686 | |
| 33 | G. S. no. 14 | 161′ 36″ | 4.95 | 16.94 | 1.31 | 434.0 | 553.8 | 348 | 206 | +515 | | 124 | | 64.82 | | 7.49 | | | | | |
| 34 | V. S. no. 8 | 162′ 34″ | | | | | | | | | 122 | | 74.09 | | 7.51 | | | | | | |
| 35 | A. S. no. 8 | 163′ 14″ | | | | | | | | | | | | | | | 86 | 1,640 | +46 | 1,686 | |
| 36 | G. S. no. 15 | 174′ 27″ | 3.73 | 17.77 | 1.21 | 340.8 | 413.5 | 273.3 | 140 | +38 | | 109 | | 56.27 | | 7.46 | | | | | |
| 37 | V. S. no. 9 | 175′ 30″ | | | | | | | | | 125 | | 56.17 | | 7.49 | | | | | | |
| 38 | A. S. no. 9 | 176′ 10″ | | | | | | | | | | | | | | | 60 | 1,307 | +65 | 1,372 | |
| 39 | G. S. no. 16 | 196′ 57″ | 2 80 | 18.44 | 1.14 | 271 5 | 308.4 | 217 8 | 91 | +54 | | | | | | | | | | | |
| 40 | V. S. no. 10 | 197′ 48″ | | | | | | | | | 133 | 115 | 46.63 | | 7.46 | | | | | | |
| 41 | A. S. no. 10 | 198′ 43″ | | | | | | | | | | | | | | | | | | | |

After 40 minutes 25 seconds of hemorrhage the blood was reinjected. Though oxygen absorption and carbon dioxide elimination increased abruptly they failed to reach the initial normal values and this despite the fact that femoral flow of blood was now greater than the initial flow. Apparently considerable deterioration of the tissues must have occurred during hemorrhage for the gaseous metabolism permanently failed to reach the normal values. The expiratory quotient diminished on reinjection just as it did on readministration of room air following anoxemia and during recovery from cyanide poisoning. The expiratory quotient, however failed to reach subnormal values. In this respect the results agree with those of the cyanide experiments but differ from those in which alveolar oxygen pressure was lowered. This absence of subnormal expiratory quotients is conceivably due to two factors working in the same direction. A damming up of carbon dioxide during hemorrhage which on reinjection of blood is free to be eliminated and an impairment of the processes of non-oxidative removal of carbon dioxide would lead to a relatively high expiratory quotient during the period of recovery. The fact that the expiratory quotient never reaches high values during hemorrhage supports the view of acid accumulation in the tissues.

The assumption of an equal distribution of lactic acid between blood and tissues may, therefore, lead to greater error in the hemorrhage experiments. Accordingly it is found that the calculated formation of lactic acid per unit of oxygen shortage was relatively small (see table 5). From hemorrhage to gas sample 3 the oxygen lactic acid ratio is 324; from gas sample 3 to 4 it was 362 and from sample 4 to 5 it was 362. With subsequent further reduction in oxidations lactic acid entered the blood stream at a more rapid rate and the lactic acid oxygen ratio dropped at gas sample 5 to 182 and at sample 6 to 193. On reinjection of the blood, lactic acid diminished along with the increase in oxidations. Although lactic acid diminished, its concentration at the end of the experiment was still above normal agreeing with the final subnormal rate of oxidations. The lactic acid oxygen ratio, 514, was high for the inclusive period of hemorrhage plus recovery. Since the corresponding rate of oxygen consumption was low, energy production was distinctly subnormal. The elimination of non-oxidative carbon dioxide during hemorrhage was relatively small. The carbon dioxide index was 0.23 but for the more inclusive period of hemorrhage plus recovery it was 0.59. It should be remembered, however, that this figure is based on removal of lactic acid by non-oxidative processes and that we have already pointed out that these processes were probably impaired during the period of recovery.

During the prehemorrhage period the hydrogen ion concentration of the arterial blood changed slowly from pH 7.24 to 7.27. With hemorrhage there is a minor and momentary alkaline swing possibly due to the greater

ventilation of the blood which has already passed through the tissues but as the blood becomes surcharged with carbon dioxide and lactic acid due to the lower rate of flow through the tissues the hydrogen ion concentration mounts. These results agree with the more common findings of Bennett (1926) and of Johnston and Wilson (1930) on the unanesthetised dog. This increase in hydrogen ion concentration continues until reinjection after which the pH is virtually constant. The checking of the rise in hydrogen ion concentration must be interpreted as a recovery phenomenon.

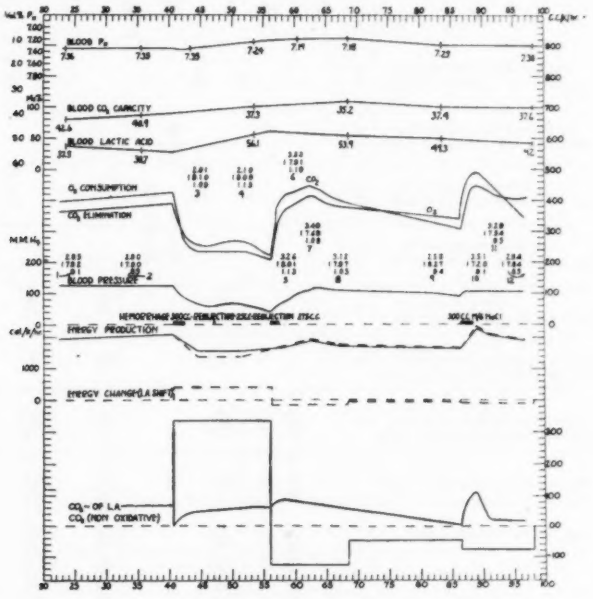



Fig. 2. Experiment 2

The changes in pH must of course be directly related to the lactic acid content and to the carbon dioxide content of the blood but why there is a lack of correspondence between blood carbon dioxide capacity and blood lactic acid is difficult to explain. Are there other fixed acids than lactic acid which combine with buffer base?

*Experiment 2.* During the initial prehemorrhage period of experiment 2 the graph shows an increase in gaseous metabolism and a decrease in blood lactic acid. This is not an uncommon result at the beginning of an experiment and is probably due to a more effective ventilation than the preceding natural ventilation. The high initial expiratory quotient, the

TABLE 2

*Experiment 2. Hemorrhage*

| OBSERVATION | PROCEDURE | TIME | PER CENT OF EXPIRED $CO_2$ | PER CENT OF EXPIRED $O_2$ | EXPIRATORY QUOTIENT | $O_2$ CONSUMPTION IN CC. PER KGM. HR. | $CO_2$ ELIMINATION IN CC. PER KGM. HR. | $CO_2$ ELIMINATION FROM OXIDATIONS BASED ON E.Q. IN CC. PER KGM. HR. | DIFFERENCE BETWEEN ACTUAL AND THEORETICAL $CO_2$ ELIMINATION | $CO_2$ EQUIVALENT OF CHANGE OF LACTIC ACID | LACTIC ACID IN MGM. PER 100 CC. ARTERIAL BLOOD | LACTIC ACID IN MGM. PER 100 CC. VENOUS BLOOD | $CO_2$ CAPACITY OF ARTERIAL BLOOD IN VOL. PER CENT | $CO_2$ CAPACITY OF VENOUS BLOOD IN VOL. PER CENT. | pH OF ARTERIAL BLOOD | pH OF VENOUS BLOOD | MEAN BLOOD PRESSURE | OXIDATIVE ENERGY IN SMALL CALORIES PER KGM. HR. | NON-OXIDATIVE ENERGY IN SMALL CALORIES PER KGM. HR. | OXIDATIVE + NON-OXIDATIVE ENERGY IN SMALL CALORIES PER KGM. HR. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12A | 12V | 13A | 13V | 14A | 14V | 15 | 16 | 17 | 18 |
| 1 | Changed T. A. | 9′ 51″ | | | | | | | | | | | | | | | 140 | | | |
| 2 | G. S. no. 1 | 22′ 21″ | 2.85 | 17.92 | 0.91 | 396 | 361.9 | 352.8 | | | | | | | | | 126 | 1,947 | | |
| 3 | A. S. no. 1 | 23′ 25″ | | | | | | | | | 37.5 | | 42.6 | | 7.36 | | | | | |
| 4 | V. S. no. 1 | 24′ 32″ | | | | | | | | | | 32.2 | | 44.3 | | 7.36 | | | | |
| 5 | G. S. no. 2 | 33′ 40″ | 2.90 | 17.80 | 0.89 | 414.1 | 368.9 | 369 | | 85 | | | | | | | 124 | 2,036 | −104 | 1,932 |
| 6 | V. S. no. 2 | 34′ 25″ | | | | | | | | | | 37.4 | | 39.3 | | 7.34 | | | | |
| 7 | A. S. no. 2 | 35′ 32″ | | | | | | | | | 30.7 | | 40.9 | | 7.35 | | | | | |
| 8 | Hem. 300 cc. | 40′ 38″ | | | | | | | | | c 28.3 | c 42 | | | | | 124 | | | |
| 9 | A. S. no. 2A | 43′ 16″ | | | | | | | | | | | | | 7.35 | | 76 | | | |
| 10 | G. S. no. 3 | 44′ 29″ | 2.01 | 19.10 | 1.09 | 233.9 | 255.5 | 208.2 | 47 | 335 | | | | | | | | 1,148 | +410 | 1,558 |
| 11 | Injection 25 cc. blood | 47′ 13″ | | | | | | | | | | | | | | | 58 | | | |
| 12 | G. S. no. 4 | 51′ 53″ | 2.10 | 19.08 | 1.15 | 233.7 | 267.6 | 208 | 60 | 335 | | | | | | | 65 | 1,147 | +410 | 1,557 |
| 13 | V. S. no. 3 | 52′ 20″ | | | | | | | | | | 58.6 | | 38.0 | | 7.24 | | | | |
| 14 | A. S. no. 3 | 53′ 30″ | | | | | | | | | 56.1 | | 37.3 | | 7.24 | | | | | |
| 15 | Reinjection of blood | 56′ 10″ | | | | | | | | | c 64.3 | c 64.3 | | | | | 40 | | | |
| 16 | G. S. no. 5 | 58′ 33″ | 3.26 | 18.01 | 1.13 | 369.7 | 418.3 | 329.3 | 89 | −126 | | | | | | | | 1,817 | −153 | 1,664 |

| | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 17 | G. S. no. 6 | 59′ 42″ | 3.33 | 17.91 | 1.10 | 383.9 | 426.1 | 342 | 84 | −126 | | | | | | | 88 | 1,885 | −153 | 1,732 |
| 18 | A. S. no. 3A | 60′ 28″ | | | | | | | | | | | | | 7.19 | | | | | |
| 19 | G. S. no. 7 | 62′ 25″ | 3.49 | 17.69 | 1.08 | 415.3 | 446.7 | 370 | 77 | −126 | | | | | | | 110 | 2,042 | −153 | 1,889 |
| 20 | G. S. no. 8 | 66′ 55″ | 3.12 | 17.97 | 1.05 | 381.0 | 399.0 | 339.3 | 60 | −126 | | | | | | | 108 | 1,873 | −153 | 1,720 |
| 21 | V. S. no. 4 | 67′ 44″ | | | | | | | | | | 60.5 | | 33.4 | | 7.19 | | | | |
| 22 | A. S. no. 4 | 68′ 32″ | | | | | | | | | 53.9 | | 35.15 | | 7.18 | | | | | |
| 23 | G. S. no. 9 | 82′ 7″ | 2.59 | 18.27 | 0.94 | 349.2 | 328.6 | 311 | 18 | −46 | | | | | | | 100 | 1,716 | −56 | 1,660 |
| 24 | V. S. no. 5 | 82′ 45″ | | | | | | | | | | 51.4 | | 35.0 | | 7.23 | | | | |
| 25 | A. S. no. 5 | 83′ 27″ | | | | | | | | | 49.3 | | 37.4 | | 7.29 | | | | | |
| 26 | Saline 500 cc. | 86′ 33″ | | | | | | | | | c 48 | c 49.2 | | | | | 90 | | | |
| 27 | G. S. no. 10 | 89′ 2″ | 3.51 | 17.20 | 0.91 | 491.3 | 446.7 | 338 | 109 | −77 | | | | | | | | 2,413 | −94 | 2,319 |
| 28 | G. S. no. 11 | 91′ 8″ | 3.29 | 17.54 | 0.95 | 443.4 | 418.8 | 395 | 24 | −77 | | | | | | | 105 | 2,180 | −94 | 2,086 |
| 29 | G. S. no. 12 | 96′ 46″ | 2.94 | 17.84 | 0.85 | 406.4 | 344.2 | 362 | 18 | −77 | | | | | | | 105 | 1,998 | −94 | 1,904 |
| 30 | V. S. no. 6 | 97′ 35″ | | | | | | | | | | 48 | | 36.8 | | 7.26 | | | | |
| 31 | A. S. no. 6 | 98′ 13″ | | | | | | | | | 42 | | 37.6 | | 7.30 | | | | | |
| 32 | Stopped drum. Changed record | | | | | | | | | | | | | | | | | | | |
| 33 | V. S. no. 7 | 110′ | | | | | | | | | | 53.7 | | V. S.-7 35.2 | | 7.28 | | | | |
| 34 | A. S. no. 7 | 110′ 30″ | | | | | | | | | 41.8 | | 38.4 | | 7.32 | | | | | |
| 35 | G. S. no. 13 | 111′ | 2.50 | 18.23 | 0.89 | 357.2 | 316.9 | 317.2 | 0 | 0 | | | | | | | | 1,758 | −3 | |

decrease in the expiratory quotient and the decrease in blood lactic acid and the decrease in the carbon dioxide capacity of the blood indicate that lactic acid is being oxidized and that accumulated carbon dioxide is being washed out and absorbed by liberated buffer base.

At 40 minutes 38 seconds—300 cc. of blood were withdrawn. This proved to be excessive and to prevent a progressive fall in mean blood pressure 25 cc. of blood were returned towards the middle of hemorrhage but even so the pressure continued to fall. At a mean blood pressure of 40 mm. Hg after a period of hemorrhage of 15 minutes 32 seconds the remaining blood was reinjected. The initial effect of hemorrhage was a sudden drop in gaseous metabolism. This was then followed by a more gradual decline. The curves of oxygen consumption and carbon dioxide elimination cross early and towards the end of hemorrhage the expiratory quotient rises to 1.15. The late increase in carbon dioxide elimination resembles results commonly obtained in the experiments with lowered alveolar oxygen and might be explained by the rapid formation of lactic acid were it not for the fact that there was an associated sudden rise in the volume flow of blood. The mid-hemorrhage injection of 25 cc. of blood is probably the cause of the augmented gaseous exchange for as the temporary increased blood flow gives way to decreasing flow there is a parallel change in gaseous metabolism. It will be noted that the curves of gaseous exchange and mean blood pressure look very much alike. Volume flow of blood shows the same changes. On reinjection of the blood, volume flow of blood and gaseous exchange were rapidly increased. While oxygen absorption never reaches the initial prehemorrhage level, carbon dioxide elimination becomes excessive and the expiratory quotient remains above unity approximately twenty minutes but then rapidly approaches the initial value. Though there is a rapid decrease in blood lactic acid the quotient fails to reach subnormal values.

At 86 minutes 33 seconds—500 cc. of saline solution were injected. Gaseous exchange increased again indicating that despite the previous reinjection of blood the volume flow of blood was not adequate to accommodate normal oxidations. This is supported by the more rapid disappearance of blood lactic acid.

In general there is a rough agreement in the blood picture as presented by the three curves of blood pH, blood carbon dioxide capacity and blood lactic acid. Barring the momentary alkaline change, the blood turns acid during hemorrhage. This increased hydrogen ion concentration is associated with an increased blood lactic acid and a decreased blood carbon dioxide capacity. Reinjection of the blood is followed by a delayed decrease in hydrogen ion concentration of the blood, a delayed increase in blood carbon dioxide capacity and a decrease in blood lactic acid. The blood samples are taken too infrequently to give accurately shaped curves.

During hemorrhage the carbon dioxide equivalent of lactic acid is large and again the amount of non oxidative-carbon dioxide eliminated is small. The carbon dioxide index is only 0.14. On reinjection of the blood there is a sudden increased elimination of non-oxidative carbon dioxide despite the relatively rapid disappearance of blood lactic acid. Assuming that our methods of computation are approximately correct elimination of non-oxidative carbon dioxide and disappearance of lactic

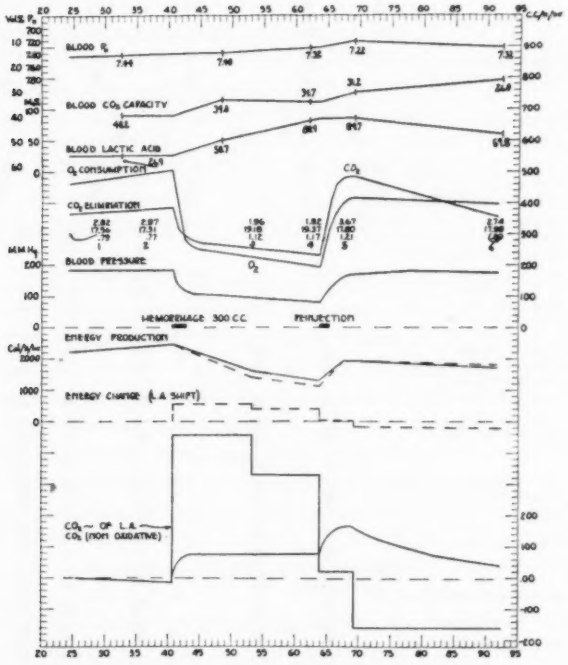



Fig. 3. Experiment 3

acid go hand in hand for the next thirty minutes. Thus if the true respiratory quotient (oxidative carbon dioxide over oxygen consumed) has remained relatively constant, which is the assumption made in these computations, then decreased flow of blood during hemorrhage would appear to cause an accumulation of acid in the tissues and subsequent increased flow an augmented elimination of this stored acid. The total end effect on the inclusive carbon dioxide index might thus approximate the inclusive

TABLE 3

*Experiment 3. Hemorrhage*

| OBSERVATION | PROCEDURE | TIME | PER CENT OF EXPIRED $CO_2$ | PER CENT OF EXPIRED $O_2$ | EXPIRATORY QUOTIENT | $O_2$ CONSUMPTION IN CC. PER KGM. HR. | $CO_2$ ELIMINATION IN CC. PER KGM. HR. | $CO_2$ ELIMINATION FROM OXIDATIONS BASED ON E.Q. IN CC. PER KGM. HR. | DIFFERENCE BETWEEN ACTUAL AND THEORETICAL $CO_2$ ELIMINATION | $CO_2$ EQUIVALENT OF CHANGE OF LACTIC ACID | LACTIC ACID IN MGM. PER 100 CC. ARTERIAL BLOOD | LACTIC ACID IN MGM. PER 100 CC. VENOUS BLOOD | $CO_2$ CAPACITY OF ARTERIAL BLOOD IN VOL. PER CENT | pH OF ARTERIAL BLOOD | MEAN BLOOD PRESSURE | OXIDATIVE ENERGY IN SMALL CALORIES PER KGM. HR. | NON-OXIDATIVE ENERGY IN SMALL CALORIES PER KGM. HR. | OXIDATIVE + NON-OXIDATIVE ENERGY IN SMALL CALORIES PER KGM. HR. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12A | 12V | 13 | 14 | 15 | 16 | 17 | 18 |
| 1 | A. S. no. 1 | 20′ 53″ | | | | | | | | | 26.6 | | | 7.46 | 184 | | | |
| 2 | V. S. no. 1 | 22′ 37″ | | | | | | | | | | 39.0 | | | | | | |
| 3 | G. S. no. 1 | 24′ 23″ | 2.82 | 17.56 | 0.79 | 461.1 | 362.8 | 363 | 0 | | | | | | | 2,233 | | |
| 4 | A. S. no. 2 | 32′ 40″ | | | | | | | | | 26.9 | | 40.2 | 7.44 | | | | |
| 5 | V. S. no. 2 | 34′ 25″ | | | | | | | | | | 39.6 | | | | | | |
| 6 | G. S. no. 2 | 36′ 30″ | 2.97 | 17.31 | 0.77 | 495.5 | 379.9 | 390 | −11 | | | | | | | 2,402 | | |
| 7 | Hem. 300 cc. | 40′ 40″ | | | | | | | | | | c 27 | | | 184 | | | |
| 8 | End of hem. | 42′ 45″ | | | | | | | | | | | | | | | | |
| 9 | A. S. no. 3 | 48′ 28″ | | | | | | | | | 50.7 | | 34.0 | 7.40 | 104 | | | |
| 10 | V. S. no. 3 | 52′ 27″ | | | | | | | | | | 62.7 | | | | | | |
| 11 | G. S. no. 3 | 53′ 13″ | 1.96 | 19.18 | 1.12 | 225.8 | 253.0 | 177 | 76 | 456 | | | | | 96 | 1,081 | +554 | 1,635 |
| 12 | V. S. no. 4 | 60′ 40″ | | | | | | | | | | 82.2 | | | | | | |
| 13 | A. S. no. 4 | 62′ 15″ | | | | | | | | | 80.9 | | 34.7 | 7.32 | | | | |
| 13A | G. S. no. 4 | 62′ 15″ | 1.82 | 19.37 | 1.17 | 199.8 | 234.4 | 157 | 77 | 329 | | | | | | 956 | +400 | 1,356 |
| 14 | Reinjection | 63′ 53″ | | | | | | | | | c 84 | | | | 80 | | | |
| 15 | G. S. no. 5 | 67′ 35″ | 3.67 | 17.80 | 1.21 | 398.4 | 479.9 | 314 | 166 | 20 | | | | | 162 | 1,930 | +24 | 1,954 |
| 16 | A. S. no. 5 | 69′ 15″ | | | | | | | | | 84.7 | | 31.2 | 7.22 | | | | |
| 17 | V. S. no. 5 | 70′ 48″ | | | | | | | | | | 97.1 | | | | | | |
| 18 | G. S. no. 6 | 91′ 48″ | 2.74 | 17.98 | 0.89 | 396.1 | 353.9 | 312 | 42 | −161 | | | | | 80′ 180 / 176 | 1,920 | −196 | 1,724 |
| 19 | A. S. no. 6 | 92′ 27″ | | | | | | | | | 59.8 | | 26.0 | 7.32 | | | | |
| 20 | V. S. no. 6 | 93′ 0″ | | | | | | | | | | 79 | | | | | | |

carbon dioxide index of the anoxemia experiments. When the total non-oxidative carbon dioxide eliminated for the period of hemorrhage and recovery is considered in relation to the corresponding carbon dioxide equivalent of lactic acid the carbon dioxide index mounts to 0.65. This is somewhat nearer the average figure for the anoxemia experiments.

The rate of lactic acid formation per unit of oxygen shortage computed on the basis of equal distribution of lactic acid in the blood and tissues is relatively low. From hemorrhage to reinjection the oxygen lactic acid ratio is 127 and from hemorrhage to gas sample 9 it is 296. The result is a drop in the total dissipation of energy.

*Experiment 3.* In this experiment the dog is bled 300 cc. between 40 minutes 40 seconds and 42 minutes 45 seconds and reinjected about twenty minutes later. The results on the whole resemble those of experiment 2 but are more striking. There is a greater reduction in oxidations during hemorrhage. There is a greater accumulation of lactic acid during hemorrhage and a greater disappearance during recovery. The expiratory quotient rises to a higher level during hemorrhage and the increase in elimination of non-oxidative carbon dioxide on reinjection is considerably more abrupt.

There is a distinct lack of correspondence between blood carbon dioxide capacity and blood lactic acid perhaps comparable to that noted by Barr, Himwich and Green (1923). The curves converge during hemorrhage and diverge during recovery. During the initial period of hemorrhage the carbon dioxide capacity of the blood diminishes parallel with the increase in blood lactic acid but later the carbon dioxide capacity seems to increase if anything during the continued rapid appearance of lactic acid in the blood. Following reinjection the carbon dioxide capacity of the blood again diminishes though the lactic acid content of the blood also decreases. This lack of correspondence between the curves of blood carbon dioxide capacity and blood lactic acid has several possible explanations. In the first place the combination of only half of the lactic acid with carbonate should produce a convergence of the curves. A passage of base into the blood stream during impaired oxidation is another possibility. A varying amount of fixed acids other than lactic acid in the blood must also be considered, and perhaps other chemical changes in the blood and tissues result in retention or elimination of carbon dioxide.

Since oxidations are greatly reduced by hemorrhage and since the amount of lactic acid formed per unit of shortage of oxygen is relatively small the curve of energy production falls below normal. Also since oxygen absorption fails to reach the initial prehemorrhage level on reinjection of blood and since our computation assumes absorption of energy by non-oxidative removal of lactic acid the curve of energy production remains below normal during the period of recovery as well.

The accumulation of lactic acid during hemorrhage is large and the carbon dioxide equivalent correspondingly large. The elimination of non-oxidative carbon dioxide, however, is small. For the period of hemorrhage the carbon dioxide index is only 0.19. On the other hand for the combined period of hemorrhage and recovery the index is 0.93. Since it is highly improbable that 93 per cent of the lactic acid combines with bicarbonate some other explanation of the high carbon dioxide index must be sought. While we have assumed for purposes of computation that the true respiratory quotient remains unchanged during the course of an acute experiment

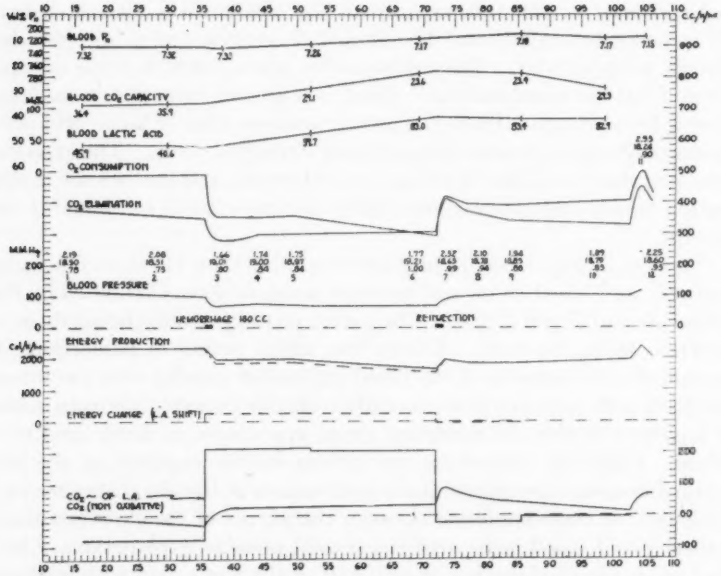



Fig. 4. Experiment 4

we have also suggested the other possibility. It is reasonable to believe that increased concentration of lactic acid, and of other reducing metabolites as well, must have some effect upon the oxidative processes as a whole. Assuming then that an accumulation of lactic acid tends towards a relatively greater oxidation of lactic acid we may picture an increased respiratory quotient. Since lactic acid accumulates enormously we have assumed a maximum increase to unity. Using this value as a new basis of computation the elimination of non-oxidative carbon dioxide now diminishes. The carbon dioxide index falls from 0.19 to 0.07 during the period of hemorrhage and from 0.93 to 0.26 for the combined period of

hemorrhage and recovery. Thus all of the computations of the carbon dioxide index in the anoxemia and cyanide experiments would also be high. Another possible explanation of the high carbon dioxide index is the possible production of fixed acids other than lactic acid. This, however, is not borne out by the carbon dioxide capacity curve.

*Experiment 4.* At 35 minutes 23 seconds the dog was bled 180 cc. and the hemorrhage was maintained for a period of 36 minutes 32 seconds. During the initial prehemorrhage period blood pH, blood carbon dioxide capacity, and oxygen consumption remained nearly constant, carbon dioxide elimination and blood lactic acid decrease indicating that the artificial ventilation was effective. The diminution of the expiratory quotient indicates that previously accumulated carbon dioxide is being washed out and that a new equilibrium is being established. The hemorrhage is relatively small. The reduction in gasesous exchange is also very small. The expiratory quotient rises accordingly at a very slow rate—from 0.80 to 0.84. During this period oxygen absorption remains virtually constant. Following gas sample 5 oxygen absorption diminishes again, carbon dioxide elimination increases and the expiratory quotient rises above normal. The break in the curve of oxygen consumption during hemorrhage and the subnormal oxygen consumption following reinjection are clearly indicative of the harmful effects of the prolonged hemorrhage. Though there is a distinct overshooting of gaseous metabolism in the sense that oxygen consumption and carbon elimination fall to a lower level soon after the abrupt increase, the overshooting falls short of the initial normal level. The early rapid fall in oxygen consumption following reinjection gives way to a prolonged but slow improvement in oxidations. At gas sample 9 oxygen absorption is 390 cc. per kgm. hour and at gas sample 10 it is 401.5 cc. Carbon dioxide elimination, however, continues to diminish indicating that the accumulated carbon dioxide is disappearing by the processes of improved ventilation (produced by increased volume flow of blood) and non-oxidative removal of lactic acid. The expiratory quotient, however, fails to reach the initial values indicating that either the true respiratory quotient has been raised by the increased concentration of lactic acid or that the carbon dioxide that was liberated and retained in the body during hemorrhage continues to be eliminated over a long period of time. The curve of non-oxidative carbon dioxide certainly indicates that carbon dioxide is being retained during the period of hemorrhage. The carbon dioxide index for this period is only 0.15 and assuming that the true respiratory quotient has risen the index is still lower. The sudden increase in elimination of non-oxidative carbon dioxide on reinjection despite the reduction in the lactic acid suggests that the transport of carbon dioxide has suddenly been improved. Despite the increased elimination of carbon

TABLE 4

*Experiment 4. Hemorrhage*

| OBSERVATION | PROCEDURE | TIME | PER CENT OF EXPIRED $CO_2$ | PER CENT OF EXPIRED $O_2$ | EXPIRATORY QUOTIENT | $O_2$ CONSUMPTION IN CC. PER KGM. HR. | $CO_2$ ELIMINATION IN CC. PER KGM. HR. | $CO_2$ ELIMINATION FROM OXIDATIONS BASED ON E.Q. IN CC. PER KGM. HR. | DIFFERENCE BETWEEN ACTUAL AND THEORETICAL $CO_2$ ELIMINATION | $CO_2$ EQUIVALENT OF CHANGE OF LACTIC ACID | LACTIC ACID IN MGM. PER 100 CC. ARTERIAL BLOOD | LACTIC ACID IN MGM. PER 100 CC. VENOUS BLOOD | $CO_2$ CAPACITY OF ARTERIAL BLOOD IN VOL. PER CENT | $CO_2$ CAPACITY OF VENOUS BLOOD IN VOL. PER CENT | pH OF ARTERIAL BLOOD | pH OF VENOUS BLOOD | MEAN BLOOD PRESSURE | OXIDATIVE ENERGY IN SMALL CALORIES PER KGM. HR. | NON-OXIDATIVE ENERGY IN SMALL CALORIES PER KGM. HR. | OXIDATIVE + NON-OXIDATIVE ENERGY IN SMALL CALORIES PER KGM. HR. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12A | 12V | 13A | 13V | 14A | 14V | 15 | 16 | 17 | 18 |
| 1 | G. S. no. 1 | 13′ 43″ | 2.19 | 18.30 | 0.78 | 485.7 | 376.8 | 377 | | | | | | | | | 118 | 2,320 | | |
| 2 | V. S. no. 1 | 15′ 19″ | | | | | | | | | | 47.6 | | 34.89 | | 7.26 | | | | |
| 3 | A. S. no. 1 | 16′ 4″ | | | | | | | | | 45.9 | | 36.37 | | 7.32 | | | | | |
| 4 | G. S. no. 2 | 27′ 41″ | 2.08 | 18.31 | 0.73 | 488 | 358.0 | 378.8 | −20 | −78 | | | | | | | 106 | 2,330 | −95 | |
| 5 | V. S. no. 2 | 28′ 54″ | | | | | | | | | | 47.7 | | 34.23 | | 7.24 | | | | |
| 6 | A. S. no. 2 | 29′ 30″ | | | | | | | | | 40.6 | | 35.92 | | 7.32 | | | | | |
| 7 | Hem. 180 cc. | 35′ 23″ | | | | | | | | | c 37.0 | c 47.8 | | | | | 100 | | | |
| 8 | G. S. no. 3 | 37′ 16″ | 1.66 | 19.01 | 0.80 | 354.8 | 284.2 | 275.2 | 9 | 212 | | | | | | | | 1,694 | +258 | 1,952 |
| 9 | A. S. no. 2A | 38′ 7″ | | | | | | | | | | | | | 7.33 | | 70 | | | |
| 10 | G. S. no. 4 | 43′ 36″ | 1.74 | 19.00 | 0.84 | 353.5 | 298.3 | 274.2 | 24 | 212 | | | | | | | 76 | 1,688 | +258 | 1,946 |
| 11 | G. S. no. 5 | 49′ 18″ | 1.75 | 18.97 | 0.84 | 357.9 | 301.1 | 277.8 | 24 | 212 | | | | | | | | 1,709 | +258 | 1,967 |
| 12 | V. S. no. 3 | 51′ 25″ | | | | | | | | | | 78.1 | | 27.81 | | 7.13 | | | | |
| 13 | A. S. no. 3 | 52′ 11″ | | | | | | | | | 59.7 | | 29.10 | | 7.26 | | 70 | | | |
| | | | | | | | | | | | | | | | | | periodic | | | |
| 14 | G. S. no. 6 | 68′ 5″ | 1.77 | 19.21 | 1.00 | 305.6 | 305.8 | 276.2 | 30 | 205 | | | | | | | 70 | 1,460 | +250 | 1,710 |
| 15 | V. S. no. 4 | 68′ 35″ | | | | | | | | | | 85.3 | | 26.76 | | 7.06 | | | | |
| 16 | A. S. no. 4 | 69′ 15″ | | | | | | | | | 83.0 | | 23.61 | | 7.17 | | | | | |
| 17 | Reinjection 180 cc. | 71′ 55″ | | | | | | | | | c 85.7 | c 86.7 | | | | | 70 | | | |

| | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 18 | G. S. no. 7 | 73′ 30″ | 2.32 | 18.65 | 0.99 | 416.3 | 411.6 | 323 | 88 | −25 | | | | | | | 92 | 1,990 | −30 1,960 |
| 19 | G. S. no. 8 | 76′ 46″ | 2.10 | 18.78 | 0.94 | 395.6 | 371.6 | 307 | 64 | −25 | | | | | | | 100 | 1,890 | −30 1,860 |
| 20 | G. S. no. 9 | 84′ 5″ | 1.94 | 18.83 | 0.88 | 390.0 | 342.6 | 302.7 | 40 | −25 | | | | | | | 108 | 1,862 | −30 1,832 |
| 21 | V. S. no. 5 | 84′ 48″ | | | | | | | | | | 88.1 | | 27.10 | | 7.19 | | | |
| 22 | A. S. no. 5 | 85′ 30″ | | | | | | | | | 83.4 | | 23.42 | | 7.10 | | | | |
| 23 | G. S. no. 10 | 97′ 1″ | 1.89 | 18.79 | 0.83 | 401.5 | 333.0 | 313 | 20 | −6 | | | | | | | 100 | 1,916 | −6 1,910 |
| 24 | V. S. no. 6 | 98′ 7″ | | | | | | | | | | 83.6 | | 27.71 | | 7.08 | | | |
| 25 | A. S. no. 6 | 98′ 45″ | | | | | | | | | 82.9 | | 29.34 | | 7.17 | | | | |
| 26 | Saline 400 cc. | 102′ 50″ | | | | | | | | | | | | | | | 100 | | |
| 27 | G. S. no. 11 | 104′ 35″ | 2.53 | 18.24 | 0.90 | 498.5 | 449.2 | 387 | 62 | | | | | | | | 115 | 2,380 | |
| 28 | A. S. | 105′ 15″ | | | | | | | | | | | | | 7.15 | | | | |
| 29 | G. S. no. 12 | 106′ 25″ | 2.25 | 18.60 | 0.93 | 428 | 399.8 | 332 | 68 | | | | | | | | | 2,042 | |

TABLE 5

*Summary of carbon dioxide indices and oxygen-lactic acid ratios*

| PERIOD | VARIATIONS IN $O_2$ CONSUMPTION CMM./KGM. (+ = EXCESS; − = SHORTAGE) | VARIATIONS IN LACTIC ACID MGM./KGM. (+ = INCREASE; − = DECREASE) | $\frac{O_2 \text{ SHORTAGE CMM./KGM.}}{\text{LACTIC ACID INCREASE MGM./KGM.}}$ | NON-OXIDATIVE $CO_2$ CC./KGM. (+ = ELIMINATION; − = RETENTION) | $CO_2$ ~ LACTIC ACID CHANGES CC./KGM. (+ = INCREASING LACTIC ACID; − = DECREASING LACTIC ACID) | $\frac{\text{NON-OXIDATIVE } CO_2}{CO_2 \sim \text{LACTIC ACID INCREASE}}$ |
|---|---|---|---|---|---|---|
| **Experiment 1** | | | | | | |
| Hem. to G. S. 3 | −6,480 | +20 | 324 | +0.00 | +5.00 | 0.00 |
| G. S. 3 to G. S. 4 | −19,920 | +55 | 362 | +2.58 | +13.75 | 0.19 |
| G. S. 4 to G. S. 5 | −17,030 | +47 | 362 | +4.13 | +11.75 | 0.35 |
| G. S. 5 to G. S. 6 | −34,460 | +188 | 182 | +10.68 | +47.00 | 0.23 |
| G. S. 6 to reinj | −19,360 | +100 | 193 | +6.20 | +25.00 | 0.25 |
| Hem. to inj. | −97,150 | +410 | 237 | +23.69 | +10.25 | 0.23 |
| Inj. to G. S. 10 | −35,910 | −225 | | +19.66 | −56.25 | |
| Hem. to G. S. 10 | −123,320 | +240 | 514 | +35.52 | +60.00 | 0.59 |
| **Experiment 2** | | | | | | |
| Hem. to reinj. | −49,690 | +368 | 127 | +13.34 | +92.00 | 0.14 |
| Hem. to G. S. 9 | −66,260 | +225 | 296 | +36.90 | +56.25 | 0.65 |

| | | | | | | |
|---|---|---|---|---|---|---|
| **Experiment 3** | | | | | | |
| Hem. to reinj. | −103,250 | +570 | 181 | +27.32 | +142.50 | 0.19 |
| Hem. to G. S. 6 | −155,750 | +332 | 469 | +77.37 | +83.00 | 0.93 |
| Using E. Q. of 1.00 | | | | | | |
| Hem. to reinj. | | | | +10.05 | +142.50 | 0.07 |
| Total period | | | | +21.44 | +83.00 | 0.26 |
| **Experiment 4** | | | | | | |
| Hem. to inj. | −90,490 | +487 | 187 | +14.39 | +121.75 | 0.15 |
| Hem. to G. S. 10 | −130,190 | +459 | 288 | +33.30 | 114.25 | 0.29 |
| **Experiment 5** | | | | | | |
| Hem. to inj. | −27,800 | +143 | 193 | +3.51 | +35.75 | 0.10 |
| 1st hem. to G. S. 9 | −15,560 | 193 | 81 | +7.39 | +48.25 | 0.15 |

dioxide the carbon dioxide index for the combined period of hemorrhage and recovery is still very low, 0.29.

Since the amount of lactic acid accumulated per unit shortage of oxygen is not very high the curve of total energy production falls during the course of hemorrhage and it fails to rise during recovery due to the subnormal rate of oxidations.

The blood picture is on the whole in very good agreement particularly during hemorrhage. During recovery the slight changes in blood pH and blood lactic acid are in better agreement with each other than with the changes in blood carbon dioxide capacity. As was previously pointed out

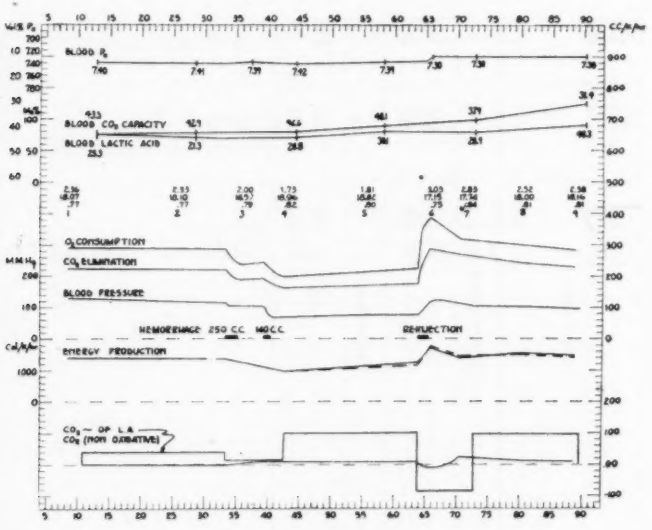



Fig. 5. Experiment 5

a convergence and divergence of the curves of blood lactic acid and blood carbon dioxide capacity is not uncommon.

*Experiment 5.* The results of this experiment differ in several ways from those of the preceding experiments. In the first place the blood lactic acid values at the outset are very low and continue to fall through the prehemorrhage period up to hemorrhage. During the extended hemorrhage there is relatively little increase in blood lactic acid. There is a slight tendency toward improvement of the subnormal oxidations indicating that the hemorrhage is well tolerated. The rise in the expiratory quotient is small. On reinjection there is a very positive overshooting of gaseous exchange and oxygen absorption remains above normal almost up to gas sample 9.

Though reinjection is accompanied by an early fall in the expiratory quotient there is a subsequent rise. The final expiratory quotient ends higher than the initial quotients before hemorrhage agreeing with the later rise in blood lactic acid. The elimination of non-oxidative carbon dioxide is again very low, much lower than in the other hemorrhage experiments. For the period of hemorrhage the carbon dioxide index was only 0.10. For the period of hemorrhage and recovery it was 0.15. As in all of the hemorrhage experiments the amount of lactic acid liberated per unit of oxygen shortage was not very great. Consequently the total energy production during hemorrhage fell off but during recovery since oxidations were augmented the energy curve rises above normal (see table 5) compensating for the deficiency in energy production during hemorrhage. For the tabulated results of experiment 5 see table 1 of the cyanide series.

## SUMMARY

The effects of hemorrhage and reinjection on various factors relating to respiration were studied during continuous and constant artificial ventilation.

Hemorrhages which decreased oxygen consumption and carbon dioxide elimination were followed by increased gaseous exchange on reinjection. A tendency towards overshooting of gaseous exchange on reinjection was present in all experiments. Absolute overshooting of the initial normal value obtained in only one experiment. The common absence of overshooting was attributed to impairment of the oxidative mechanism of the tissue cells during hemorrhage.

The relatively greater decrease in oxygen absorption as compared with carbon dioxide elimination during hemorrhage resulted in an increase of the expiratory quotient. This increase on the whole was less than that observed during impaired oxidations with lowered alveolar oxygen.

Reinjection of blood decreased the expiratory quotient but never to subnormal levels. This differs from the effects of lowered alveolar oxygen but agrees more closely with the effects of intravenous injection of sodium cyanide.

The impaired oxidations of hemorrhage were associated with accumulation of lactic acid in the blood. The greater the impairment of oxidations the greater the accumulation of lactic acid in the blood. The improved oxidations of reinjection were accompanied by a disappearance of lactic acid in the blood or by a slower accumulation. The amount of lactic acid accumulating in the blood per unit of oxygen shortage was less than in either of the two preceding series of experiments.

The ratio of non-oxidative carbon dioxide eliminated to the carbon dioxide equivalent of lactic acid during hemorrhage was very much

smaller than during impaired oxidation from lowered alveolar oxygen. For the period of injection the proportion was larger.

The small carbon dioxide index of hemorrhage was explained on the basis of acid accumulation in the tissues during hemorrhage and the subsequent large carbon dioxide index of reinjection was explained by the freer elimination of this stored acid.

The small increase in the expiratory quotient of hemorrhage and the small decrease with reinjection was similarly explained.

Reasons are given for believing that the true or metabolic respiratory quotient of the tissues during the short periods of hemorrhage are constantly changing. This should hold for altered oxidations produced by other methods as well.

The curves of carbon dioxide capacity (inverted curve) and lactic acid content of the blood were not always roughly parallel. The convergence of the curves during hemorrhage and divergence during reinjection agree with the partial reaction of lactic with bicarbonate.

The relatively small accumulation of lactic acid in the blood per unit of oxygen shortage agrees with the suggestion that acid accumulates in the tissues during hemorrhage.

This smaller accumulation of blood lactic acid per unit of oxygen shortage explains the decrease in total energy liberated during hemorrhage as indicated by the computed curves of energy dissipation.

The accumulation of lactic acid in the tissues due to impaired removal probably prevents a continuous and rapid formation of lactic acid which in turn would limit this anaerobic source of energy.

## BIBLIOGRAPHY

BARR, HIMWICH AND GREEN. 1923. Journ. Biol. Chem., lv, 495.
BENNETT. 1926. Journ. Biol. Chem., lxix, 675
HERTZMAN AND GESELL. 1927. This Journal, lxxxi, 563.
JOHNSTON AND WILSON. 1930. Journ. Biol. Chem., lxxxv, 727.
RIEGEL. 1927. Journ. Biol. Chem., lxxiv, 123.

# THE REGULATION OF RESPIRATION

## A Study of the Correlation of Numerous Factors of Respiratory Control Following Intravenous Injection of Sodium Bicarbonate[1,2]

ROBERT GESELL, HUGO KRUEGER,[3] GRACE GORHAM AND THEODORE BERNTHAL

*From the Department of Physiology, University of Michigan, Ann Arbor*

Received for publication March 17, 1930

That intravenous injection of sodium bicarbonate temporarily increases mean blood pressure and respiratory movements and that similar injection of sodium carbonate has opposite effects is a well known observation. That the diametrically opposite action is possibly attributable to opposite effects on the carbon dioxide pressure of the blood is now supported by experimental data (Gesell, 1923; Gesell and Hertzman, 1926; Gesell and McGinty, 1927). The present series of papers constitutes another effort at a closer analysis of the acid base disturbances associated with administration of sodium bicarbonate. For this purpose we have used the methods of the preceding experiments.

Results. *Experiment 1.* (See fig. 1 and for an example of a smoked record obtained with the continuous methods see fig. 5 of this paper, fig. 96, Gesell, 1929, and fig. 1, Gesell, 1928.) Prior to injection of sodium bicarbonate the condition of the animal is relatively constant considering the initial low blood pressure. While oxygen consumption and mean blood pressure are slowly falling, blood pH, blood carbon dioxide capacity, blood lactic acid and carbon dioxide elimination are nearly constant. With the injection of molecular sodium bicarbonate beginning at 42 minutes 28 seconds and lasting roughly five minutes, a number of chemical changes are initiated. First of all there is a sudden decrease in the hydrogen ion concentration of the blood from pH 7.03 to pH 7.34. This lowered hydrogen ion concentration outlasts the period of injection and only at 102 minutes 2 seconds does the blood begin to show a distinct change in the

[1] Preliminary report—This Journal, 1929 xc, 360.

[2] This investigation has been made with the assistance of a grant from the Committee on Therapeutic Research, Council on Pharmacy and Chemistry, American Medical Association.

[3] Submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

acid direction. At that point the blood, although more acid than normal, is still 0.24 pH more alkaline than the preadministration level. This alkaline change is accompanied by an increase in the carbon dioxide capacity of the blood from 27.6 volumes per cent to 61.3 volumes per cent. The carbon dioxide capacity then diminishes but at a relatively slow rate. The immediate effect of bicarbonate on blood lactic acid is a pronounced increase followed by a temporary decrease and a final increase. Decreasing oxygen consumption changes abruptly to increasing oxygen absorption

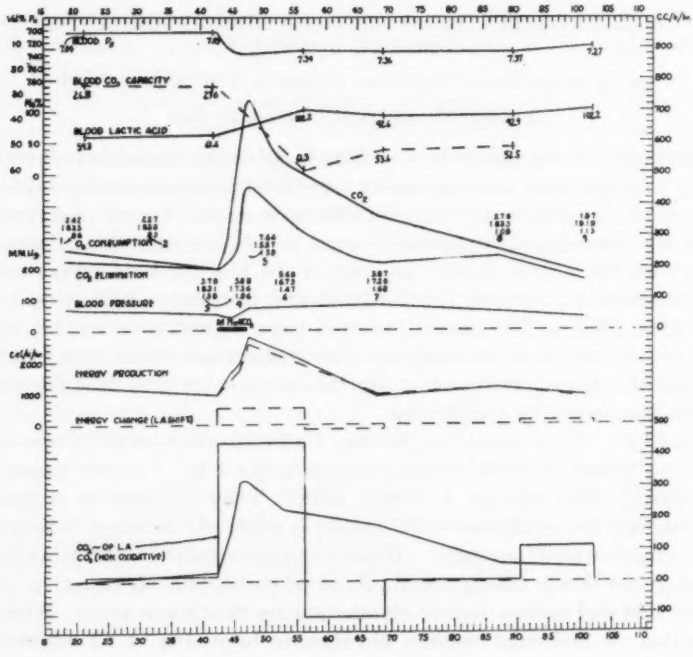



Fig. 1. Experiment 1

from 224.2 cc. to 461.8 cc. per kgm. hour. The crest of the curve is sharp. The maximum rate is of short duration giving way to progressive reduction. Carbon dioxide elimination increases even more. It rises from 209 cc. to 733.8 cc. per kgm. hour or about three and half times the preadministration rate. The expiratory quotient rises from 0.93 to 1.96 indicating a high output of non-oxidative carbon dioxide. The maximum carbon dioxide elimination is also of very short duration. Barring the temporary reduction in mean blood pressure, the general effect is a well sustained elevation in pressure which falls again along a gradient parallel with the preadminis-

TABLE 1

*Experiment 1. Sodium bicarbonate*

| OBSERVATION | PROCEDURE | TIME | PER CENT OF EXPIRED $CO_2$ | PER CENT OF EXPIRED $O_2$ | EXPIRATORY QUOTIENT | $O_2$ CONSUMPTION IN CC. PER KGM. HR. | $CO_2$ ELIMINATION IN CC. PER KGM. HR. | $CO_2$ ELIMINATION FROM OXIDATIONS BASED ON E.Q. IN CC. PER KGM. HR. | DIFFERENCE BETWEEN ACTUAL AND THEORETICAL $CO_2$ ELIMINATION | $CO_2$ EQUIVALENT OF CHANGE OF LACTIC ACID | LACTIC ACID IN MGM. PER 100 CC. ARTERIAL BLOOD | LACTIC ACID IN MGM. PER 100 CC. VENOUS BLOOD | $CO_2$ CAPACITY OF ARTERIAL BLOOD IN VOL. PER CENT | $CO_2$ CAPACITY OF VENOUS BLOOD IN VOL. PER CENT | pH OF ARTERIAL BLOOD | pH OF VENOUS BLOOD | MEAN BLOOD PRESSURE | OXIDATIVE ENERGY IN SMALL CALORIES PER KGM. HR. | NON-OXIDATIVE ENERGY IN SMALL CALORIES PER KGM. HR. | OXIDATIVE + NON-OXIDATIVE ENERGY IN SMALL CALORIES PER KGM. HR. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12A | 12V | 13A | 13V | 14A | 14V | 15 | 16 | 17 | 18 |
| 1 | G. S. no. 1 | 18′ 10″ | 2.42 | 18.25 | 0.86 | 258.8 | 222.2 | 222.2 | | | | | | | | | 68 | 1,261 | | |
| 2 | V. S. no. 1 | 19′ 45″ | | | | | | | | | | 66.6 | | 28.71 | | 6.93 | | | | |
| 3 | A. S. no. 1 | 21′ 30″ | | | | | | | | | 59.3 | | 26.83 | | 7.04 | | 64 | | | |
| 4 | G. S. no. 2 | 31′ 15″ | 2.27 | 18.58 | 0.93 | 224.2 | 209.0 | 192.7 | 16 | +17 | | | | | | | 60 | 1,095 | | |
| 5 | V. S. no. 2A | 37′ 42″ | | | | | | | | | | | | | | | | | | |
| 6 | V. S. no. 2 | 39′ 40″ | | | | | | | | | | 72.4 | | 28.68 | | 6.98 | | | | |
| 7 | A. S. no. 2 | 41′ 59″ | | | | | | | | | 61.6 | | 27.62 | | 7.03 | | | | | |
| 8 | m $NaHCO_3$ | 42′ 28″ | | | | | | | | | c 61.6 | c 73.8 | | | | | 51 | | | |
| 9 | G. S. no. 3 | 44′ 30″ | 3.70 | 18.31 | 1.58 | 224.7 | 347.8 | 193 | 155 | +441 | | | | | | | | 1,096 | +536 | 1,632 |
| 10 | G. S. no. 4 | 46′ 3″ | 5.88 | 17.36 | 1.96 | 287.8 | 564.1 | 247.2 | 317 | +441 | | | | | | | | 1,402 | +536 | 1,938 |
| 11 | G. S. no. 5 | 47′ 28″ | 7.64 | 15.57 | 1.59 | 461.8 | 733.8 | 396.8 | 337 | +441 | | | | | | | 70 | 2,250 | +536 | 2,786 |
| 12 | G. S. no. 6 | 53′ 5″ | 5.68 | 16.73 | 1.47 | 365.9 | 539.0 | 313.2 | 226 | | | | | | | | 73 | 1,782 | | |
| 13 | V. S. no. 3 | 54′ 36″ | | | | | | | | | | 102.4 | | 63.98 | | 7.27 | | | | |
| 14 | A. S. no. 3 | 56′ 17″ | | | | | | | | | 102.2 | | 61.28 | | 7.34 | | | | | |
| 15 | G. S. no. 7 | 67′ 48″ | 3.87 | 17.28 | 1.68 | 214.6 | 360.3 | 184.4 | 176 | −113 | | | | | | | 80 | 1,046 | −138 | 908 |
| 16 | V. S. no. 4 | 68′ 23″ | | | | | | | | | | 91.9 | | 53.1 | | 7.34 | | | | |
| 17 | A. S. no. 4 | 69′ 0″ | | | | | | | | | 92.6 | | 53.6 | | 7.36 | | | | | |
| 18 | G. S. no. 8 | 87′ 27″ | 2.78 | 18.33 | 1.09 | 236.5 | 257.8 | 203.1 | 55 | +2 | | | | | | | 58 | 1,153 | +4 | 1,157 |
| 19 | V. S. no. 5 | 88′ 48″ | | | | | | | | | | 89.2 | | 46.82 | | 7.29 | | | | |
| 20 | A. S. no. 5 | 89′ 42″ | | | | | | | | | 92.9 | | 52.45 | | 7.37 | | | | | |
| 21 | G. S. no. 9 | 101′ 4″ | 1.97 | 19.19 | 1.13 | 160.8 | 181.4 | 138.1 | 43 | +110 | | | | | | | 40 | 784 | +134 | 918 |
| 22 | V. S. no. 6 | 101′ 55″ | | | | | | | | | | 95.6 | | 42.65 | | 7.20 | | | | |
| 23 | A. S. no. 6 | 102′ 23″ | | | | | | | | | 102.2 | | | | 7.27 | | | | | |

tration fall in pressure. The initial effects of bicarbonate on respiratory movements are similar to those on mean blood pressure. Glancing next at the curves of the carbon dioxide equivalent of lactic acid and non-oxidative carbon dioxide the high proportion of non-oxidative carbon dioxide is evident. For the period beginning with the injection of bicarbonate and ending with gas sample 8 the non-oxidative carbon dioxide is considerably greater than the computed carbon dioxide equivalent of the lactic acid which is formed. The carbon dioxide index is 1.65. Our premise is thus supported that there are several factors accounting for augmented elimination of carbon dioxide following bicarbonate injection.

The curve of energy production shows an increase from 1095 calories per kgm. hour to 2786, a value approximately two and one-half times the preadministration rate. This enormous increase in energy dissipation must be considered as one of the outstanding effects of intravenous injection of sodium bicarbonate.

*Experiment 2.* (See table 1 of the hemorrhage series.) Two hundred cubic centimeters of molecular sodium bicarbonate were injected at 152 minutes 1 second over a period of approximately five minutes. The previous history of the animal is 300 cc. saline injection at 0 minute, 260 cc. hemorrhage at 54 minutes 5 seconds, 25 cc. reinjection at 58 minutes 27 seconds and 245 cc. reinjection at 94 minutes 30 seconds. One-half hour after the saline injection the mean blood pressure is 140 mm. Hg. At gas sample 11 (the first in the graph) the pressure is down to 88 mm. Hg. The corresponding figures for oxygen consumption (see table corresponding to experiment 1 in the hemorrhage series, p. 369) at the beginning of the experiment and at gas sample 11 are 451.8 cc. and 316.7 cc. per kgm. hour. Lactic acid increased from 37.6 mgm. to 78.1 per 100 cc. of blood. This general deterioration is seen in the gradient of the curves during the preadministration period (see graph corresponding to experiment 1 of the hemorrhage series, p. 366). Lactic acid is increasing, oxygen consumption and carbon dioxide elimination are decreasing, mean blood pressure is falling and blood pH is nearly constant. These conditions are suddenly changed by the injection of bicarbonate. The blood pH increases from 7.15 to 7.51. The carbon dioxide capacity of the blood increases from 22.6 volumes per cent to 74.1 volumes per cent. Oxygen consumption increases 44 per cent from 316.7 cc. to 457.2 cc. per kgm. hour. Carbon dioxide elimination increases 150 per cent from 270 cc. to 675.3 cc. per kgm. hour. The respiratory quotient increases from 0.85 to 1.70 at gas sample 12. These rapid changes last approximately through the period of injection and then are reversed but at a slower rate. The hydrogen ion concentration of the blood increases at a very slow rate ending at a pH of 7.46. Oxygen consumption falls off more abruptly and eventually reaches the preadministration value. Carbon dioxide elimination

decreases but remains above the rate of oxygen consumption. The curves of carbon dioxide elimination and oxygen absorption converge but at gas sample 16 the expiratory quotient is still above unity—1.14. Lactic acid increases—rapidly at first and more slowly towards the end. Respiratory movements are markedly stimulated during the period of higher elimination of carbon dioxide after which they diminish progressively as the animal deteriorates. Comparable effects are seen on mean blood pressure.

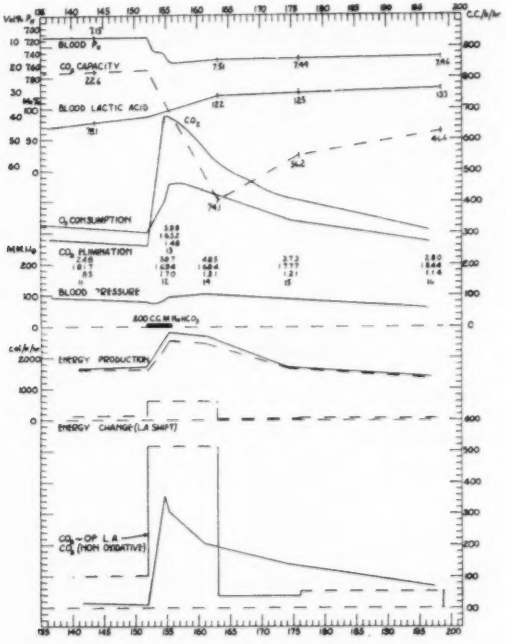



Fig. 2. Experiment 2

The carbon dioxide equivalent of lactic acid which accumulates following the injection of bicarbonate up to gas sample 16 is 116.5 cc. Non-oxidative carbon dioxide for the same period is 114.5 cc. Dividing the latter by the former another high carbon dioxide index is obtained indicating that 98 per cent of the lactic acid formed combined with bicarbonate. The results of the preceding experiment are, therefore, supported. The same abrupt changes in energy production occurred as in experiment 1.

*Experiment 3.* (See table 2 of the cyanide series.) Sodium bicarbonate was administered late in the experiment. The previous history is injection of sodium cyanide at 27 minutes 23 seconds and at 37 minutes 32 seconds.

The mean blood pressure at 0 minute was 115 mm. Hg and at 121 minutes when the bicarbonate was injected it was 76 mm. Hg. Oxygen consumption fell from 490.6 cc. to 316.9 cc. per kgm. hour. Carbon dioxide elimination fell from 380.8 cc. to 292.5 cc. per kgm. hour. Lactic acid rose from 27.8 mgm. to 67.6 mgm. per 100 cc. of blood and the expiratory quotient rose from 0.78 to 0.92. Injection was, therefore, administered to a deteriorated animal. There was the usual decrease in hydrogen ion concentration of blood accompanied by increased carbon dioxide capacity and

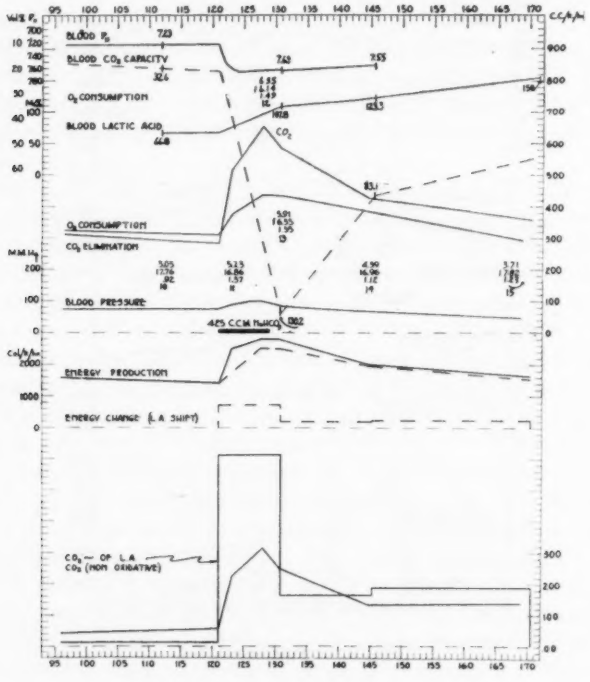



Fig. 3. Experiment 3

lactic acid content of the blood. Oxygen consumption was increased but not to the rate of 490.6 cc. per kgm. hour which obtained at gas sample 1 at the beginning of the experiment. Carbon dioxide eliminated at gas sample 12 exceeded the initial rate and the expiratory quotient rose to 1.49. From gas sample 12 to gas sample 14 the gaseous exchange declined with a drop in the expiratory quotient to 1.12. Gaseous exchange diminished still further between gas samples 14 and 15 but the expiratory quotient rose to 1.23. It will be seen that this rise is associated with a

rapid accumulation of lactic acid. Although lactic acid accumulates at a rapid rate after the injection of bicarbonate the consecutive changes in concentration of lactic acid in the blood are of a similar kind as in the other three experiments. This is best brought out by the curve of carbon dioxide equivalent of lactic acid. In experiment 1 after the initial lactic acid increase there is an actual decrease, then a period of constant concentration, then a final period of increasing concentration. These same three

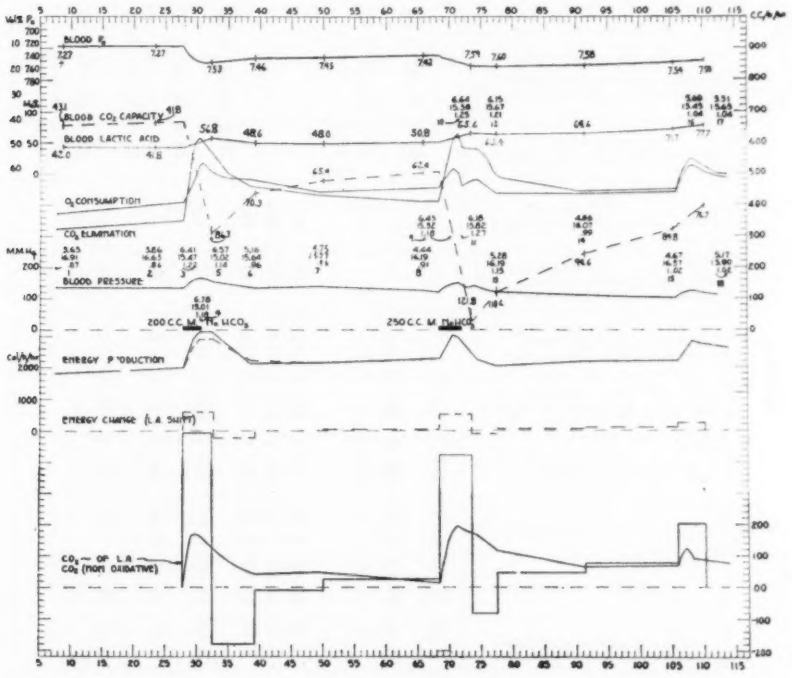



Fig. 4. Experiment 4

jogs in the curve are seen in experiment 2 although here an absolute decrease never occurs. There is only a relative decrease. Despite the more rapid increase in lactic acid in experiment 3 the three jogs are still clearly evident. In experiment 4 the lactic acid changes resemble more closely those of experiment 1.

Although the total energy production is again enormously augmented in experiment 3 by injection of bicarbonate it fails to reach the level of dissipation at the beginning of the experiment.

TABLE 2

*Experiment 4. Sodium bicarbonate*

| OBSERVATION | PROCEDURE | TIME | PER CENT OF EXPIRED $CO_2$ | PER CENT OF EXPIRED $O_2$ | EXPIRATORY QUOTIENT | $O_2$ CONSUMPTION IN CC. PER KGM. HR. | $CO_2$ ELIMINATION IN CC. PER KGM. HR. | $CO_2$ ELIMINATION FROM OXIDATIONS BASED ON E.Q. IN CC. PER KGM. HR. | DIFFERENCE BETWEEN ACTUAL AND THEORETICAL $CO_2$ ELIMINATION | $CO_2$ EQUIVALENT OF CHANGE OF LACTIC ACID | LACTIC ACID IN MGM. PER 100 CC. ARTERIAL BLOOD | $CO_2$ CAPACITY OF ARTERIAL BLOOD IN VOL. PER CENT | pH OF ARTERIAL BLOOD | MEAN BLOOD PRESSURE | OXIDATIVE ENERGY IN SMALL CALORIES PER KGM. HR. | NON-OXIDATIVE ENERGY IN SMALL CALORIES PER KGM. HR. | OXIDATIVE + NON-OXIDATIVE ENERGY IN SMALL CALORIES PER KGM. HR. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 |
| 1 | G. S. no. 1 | 7′ 21″ | 3.63 | 16.91 | 0.87 | 371.5 | 321.5 | | | | | | | 134 | 1,814 | | |
| 2 | A. S. no. 1 | 8′ 30″ | | | | | | | | | 42.0 | 43.05 | 7.27 | | | | |
| 3 | G. S. no. 2 | 22′ 36″ | 3.86 | 16.63 | 0.86 | 397.6 | 342.1 | 344 | −2 | | | | | 134 | 1,942 | | |
| 4 | A. S. no. 2 | 23′ 20″ | | | | | | | | | 41.8 | 41.83 | 7.27 | | | | |
| 5 | M/$NaHCO_3$, 200 cc. | 27′ 48″ | | | | | | | | | c 41.7 | | | 133 | | | |
| 6 | G. S. no. 3 | 29′ 30″ | 6.41 | 15.47 | 1.22 | 476.4 | 580.7 | 412 | 169 | 494 | | | | | 2,328 | +601 | 2,927 |
| 7 | G. S. no. 4 | 30′ 25″ | 6.78 | 15.01 | 1.18 | 520.7 | 613.4 | 450.5 | 163 | 494 | | | | 165 | 2,541 | +601 | 3,142 |
| 8 | G. S. no. 5 | 31′ 24″ | 6.57 | 15.02 | 1.14 | 522.4 | 593.3 | 452 | 141 | 494 | | | | | 2,551 | +601 | 3,152 |
| 9 | A. S. no. 3 | 32′ 23″ | | | | | | | | | 56.8 | 86.29 | 7.53 | 155 | | | |
| 10 | G. S. no. 6 | 38′ 26″ | 5.16 | 15.64 | 0.96 | 480.6 | 460.4 | 415.6 | 45 | −180 | | | | | 2,347 | −218 | 2,129 |
| 11 | A. S. no. 4 | 39′ 14″ | | | | | | | | | 48.6 | 70.31 | 7.46 | 133 | | | |
| 12 | G. S. no. 7 | 49′ 20″ | 4.75 | 15.77 | 0.98 | 440.9 | 429.8 | 381 | 49 | −8 | | | | | 2,152 | −10 | 2,142 |
| 13 | A. S. no. 5 | 50′ 9″ | | | | | | | | | 48.0 | 65.43 | 7.45 | 138 | | | |
| 14 | G. S. no. 8 | 65′ 15″ | 4.44 | 16.19 | 0.91 | 453.4 | 411.4 | 392.2 | 19 | +26 | | | | | 2,212 | +32 | 2,244 |
| 15 | A. S. no. 6 | 65′ 57″ | | | | | | | | | 50.8 | 62.39 | 7.42 | 126 | | | |
| 16 | $NaHCO_3$, 250 cc. | 68′ 30″ | | | | | | | | | c 51.2 | | | 120 | | | |

| | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 17 | G. S. no. 9 | 70′ 37″ | 6.43 | 15.32 | 1.18 | 514.0 | 605.9 | 424.6 | 181 | +423 | | | | | 2,510 | +518 | 3,028 |
| 18 | G. S. no. 10 | 71′ 21″ | 6.64 | 15.38 | 1.25 | 502.8 | 628.2 | 435 | 193 | +423 | | | | 150 | 2,455 | +518 | 2,973 |
| 19 | G. S. no. 11 | 72′ 6″ | 6.18 | 15.82 | 1.27 | 461.4 | 584.3 | 399.2 | 185 | +423 | | | | | 2,251 | +518 | 2,769 |
| 20 | A. S. no. 7 | 73′ 35″ | | | | | | | | | 65.6 | 121.82 | 7.59 | | | | |
| 21 | G. S. no. 12 | 74′ 28″ | 6.15 | 15.67 | 1.21 | 479.6 | 579.8 | 414.7 | 165 | −83 | | | | 140 | 2,341 | −100 | 2,241 |
| 22 | G. S. no. 13 | 77′ 26″ | 5.28 | 16.19 | 1.13 | 437.3 | 494.9 | 378.2 | 117 | −83 | | | | | 2,135 | −100 | 2,035 |
| 23 | A. S. no. 8 | 77′ 35″ | | | | | | | | | 63.4 | 110.64 | 7.60 | 124 | | | |
| 24 | G. S. no. 14 | 90′ 58″ | 4.86 | 16.07 | 0.99 | 435.9 | 440.4 | 376 | 64 | +47 | | | | 110 | 2,122 | +57 | 2,179 |
| 25 | A. S. no. 9 | 91′ 26″ | | | | | | | | | 64.6 | 94.63 | 7.58 | | | | |
| 26 | G. S. no. 15 | 105′ 13″ | 4.67 | 16.37 | 1.02 | 441 | 448.6 | 381.2 | 67 | 75.4 | | | | 104 | 2,152 | +92 | 2,244 |
| 27 | A. S. no. 10 | 105′ 28″ | | | | | | | | | 71.7 | 84.78 | 7.54 | | | | |
| 28 | Saline 550 cc. | 106′ 0″ | | | | | | | | | c 71.8 | | | | | | |
| 29 | G. S. no. 16 | 108′ 20″ | 5.69 | 15.45 | 1.04 | 527.7 | 547.2 | 456 | 91 | 202 | | | | 125 | 2,573 | +246 | 2,819 |
| 30 | G. S. no. 17 | 109′ 40″ | 5.51 | 15.63 | 1.04 | 511.1 | 530.1 | 442 | 88 | 202 | | | | | 2,496 | +246 | 2,742 |
| 31 | A. S. no. 11 | 110′ 18″ | | | | | | | | | 77.7 | 76.56 | 7.50 | 118 | | | |
| 32 | G. S. no. 18 | 114′ | 5.17 | 15.90 | 1.02 | 485.4 | 496.1 | 420 | 76 | | | | | | 2,370 | +246 | 2,616 |

Experiment 4 shows the results of two successive injections of sodium bicarbonate. While the effects of these injections are accumulative with

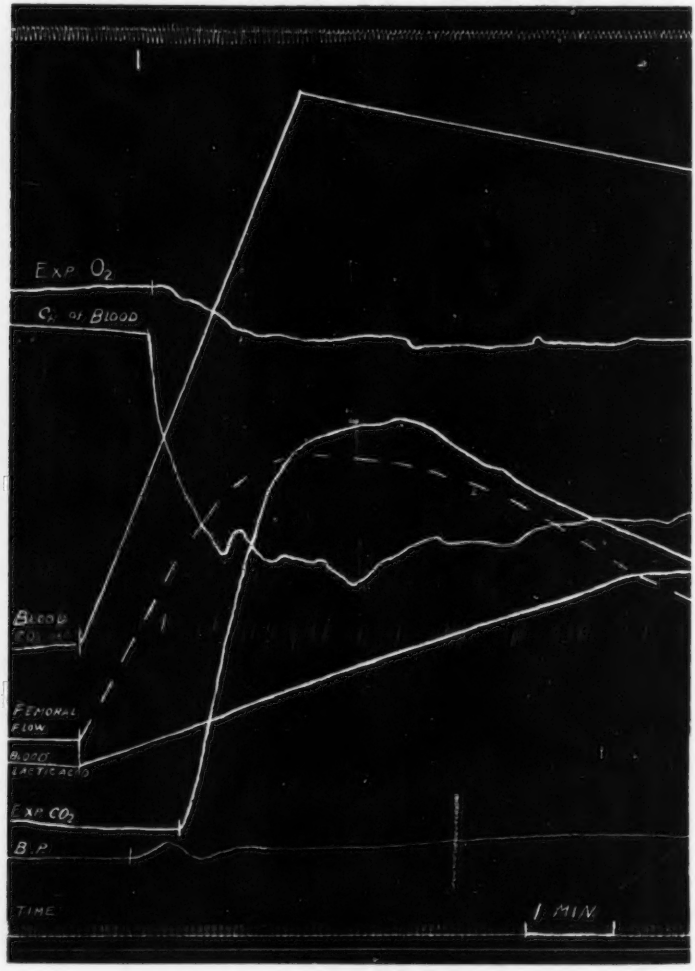



Fig. 5

respect to hydrogen ion concentration and lactic acid content of the blood they are not as additive with respect to gaseous exchange. The second injection to be sure increases the gaseous exchange but it does not raise

the general level as the first injection did. The high level of gaseous exchange, however, is maintained. In that connection the general fall in blood pressure is of interest. The rapid post-injectional removal of lactic acid in both observations and the general similarity of the curves of carbon dioxide equivalent of lactic acid removal are also of interest. The ratios of carbon dioxide equivalent of lactic acid to non-oxidative carbon dioxide are very high again, with the first injection the ratio is 1.64 and with the second the ratio is 1.12. Obviously there is some important source of carbon dioxide elimination other than the formation of lactic acid.

TABLE 3

*Summary of acid base equilibrium*

| | | EXPERIMENT | | | | | |
|---|---|---|---|---|---|---|---|
| | | 2 | 3 | 4a | 4b | 5 | 6 |
| 1 | $CO_2 \backsim NaHCO_3$ injected in cc. per kgm. | 217.5 | 453.3 | 196.4 | 245.6 | 270.6 | 256.3 |
| 2 | Non-oxidative $CO_2$ in cc. per kgm. | 114.47 | 137.7 | 36.87 | 57.93 | 60.74 | 44.43 |
| 3 | $CO_2 \backsim$ L. A. in cc. per kgm. | 116.5 | 211.0 | 22.5 | 51.75 | 110 | 41.5 |
| 4 | Per cent of L. A. effective in driving off $CO_2$ | 98 | 65 | 164 | 112 | 55 | 107 |
| 5 | Non-oxidative $CO_2/CO_2 \backsim NaHCO_3$ injected | 52.6 | 33 | 19 | 22 | 22 | 17 |
| 6 | $CO_2 \backsim$ L. A./$CO_2 \backsim NaHCO_3$ injected | 53.6 | 46.5 | 11 | 21 | 41 | 16 |
| 7 | $CO_2$ capacity of blood before injecting $NaHCO_3$ in vol. per cent | 22 | 33.75 | 41.5 | 61.75 | 34 | 27.75 |
| 8 | $CO_2$ capacity of blood after injecting $NaHCO_3$ in vol. per cent | 46.63 | 69.00 | 61.75 | 85.00 | 56.24 | 61.90 |
| 9 | $CO_2$ capacity of increase in vol. per cent | 24.63 | 35.25 | 20.25 | 23.75 | 22.24 | 34.15 |
| 10 | $CO_2$ capacity increase expected | 21.75 | 45.33 | 19.64 | 24.56 | 27.06 | 25.63 |
| 11 | $NaHCO_3$ disappearing from the blood in vol. per cent | | 10.08 | | 0.81 | 4.82 | |

The effects of both injections on respiratory movements are exceptionally striking. Before the first injection the animal was not quite apneic but very nearly so. Injection augmented the respiratory movements which reached their maximum at the peak of the curve of carbon dioxide elimination. Then the movements diminished and half way between gas samples 6 and 7 the animal was apneic. The second injection produced a second set of respiratory movements which were maximal at the height of carbon dioxide elimination. At 84 minutes the dog was apneic again.

The coincidence of similar changes in blood pressure and respiratory movement supports the view of a common chemical regulation of pulmonary ventilation and circulation. A record of the changes of respiratory movements associated with intravenous injection of sodium bicarbonate is seen in figure 5 which corresponds to the period between observation 25 and 33 of table 3 of the following paper. The momentary augmentation associated with high carbon dioxide pressure of the expired air is quite obvious. So is the subsequent depression of respiratory movements below the preinjection level which is a common finding. It is thus of interest to know the fate and distribution of the bicarbonate injected. While our data will not permit an exact analysis, inasmuch as the excretion of bicarbonate by the kidneys was not followed, the appended table 3 is nevertheless suggestive. Assuming an equal distribution of bicarbonate between the blood and tissues we have computed the expected increase in the blood (line 11) compared that with the observed increase (line 10) in six experiments. On the whole the results would indicate a relatively rapid movement of bicarbonate from the blood stream suggesting that the late effect of intravenous injections of bicarbonate is not only an increased alkalinity of the blood but of the tissues as well. It would thus appear that the initial acid effect of bicarbonate may change to a later alkaline effect due to a migration of base.

Discussion. While these experiments have brought out various effects of intravenous injection of sodium bicarbonate in a more quantitative way than our earlier experiments they still leave many of our queries open to speculation.

A number of years ago Macleod and others (Macleod and Hoover, 1917; Macleod, 1918; Macleod and Knapp, 1918; Macleod, 1921; Anrep and Cannon, 1923) called attention to the increase in lactic acid of the blood associated with intravenous injection of sodium bicarbonate and the compensating acid effects on the blood of this migration of acid from the tissues. But what is the mechanism of this migration? Does it lead to depletion of lactic acid in the tissues or does the increase in lactic acid occur throughout the body? Does lactic acid migrate as undissociated acid or does it migrate as a result of ionic exchange between the blood and tissues? Is the increase in rate of oxidation due to increased cellular lactic acid or is it due to decreased hydrogen ion concentration in the cell or only at the cell membrane?

It would seem that the nature of the migration of lactic acid is of fundamental importance. If sodium bicarbonate does not enter the cell and if the increased alkalinity of the external environment causes increased liberation of cellular lactic acid then the interior of the cell might turn more acid. In that event oxidations might increase by virtue of the increased concentration of combustible material, if they were not in turn inhibited

by increased intracellular hydrogen ion concentration. Lactic acid might thus migrate from the tissues to the blood in the undissociated form.

If on the other hand lactic acid migrates as a result of exchange of anions between the cell and its environment the chain of events may be very different. Let us imagine a diffusion of $HCO'_3$ ions from the blood into the tissues as a result of increased pressure of these ions in the blood and that these ions replace the lactate anion which then migrates from the cell to the external environment and into the blood. Since lactic acid is a stronger acid than carbonic acid this exchange is in favor of increased hydrogen ion concentration in the blood and decreased hydrogen ion concentration in the tissues. This exchange by itself would, therefore, have two effects on the cell—a depletion of lactic acid and a decrease in free hydrogen ions. But the lactic acid equilibrium in the cell may be immediately reëstablished by the formation of new lactic acid. In this connection we should recall the work of Meyerhoff showing that the concentration of lactic acid in muscle brei is augmented by a decrease in hydrogen ion concentration. A mechanism may, therefore, be at hand permitting replenishment of lactic acid which has left the cell. If to this complete replenishment of lactic acid we add the possibility of slow migration of sodium bicarbonate across the cell membrane with its subsequent neutralization by further formation of lactic acid a super accumulation of lactic acid would be explained. It should be remembered that each accumulation of lactic acid in the blood and immediate cell environment would lead to greater concentration of lactic acid in the cell by virtue of the back pressure exerted. This obviously contributes to increasing cellular acidity. The general increase in lactic acid in turn most probably contributes towards hyperoxidations with excessive formation of carbon dioxide.

How all of these hypothetical factors might cancel out in the end effect on cellular acidity is speculative. More direct experiments are called for, but taking the changes step by step it is obvious that the first effect of displacement of the lactate anion by the $HCO'_3$ ion from the blood would decrease cellular acidity and increase blood acidity. The next replacement of the $HCO'_3$ anion in the cell by newly formed lactic acid should be an acid effect for the buffer base is now diminished and the free acid increased. The net result of the initial alkaline and the subsequent acid effect might thus be a very small change. Complete reaction of the slowly migrating sodium bicarbonate with newly formed intracellular lactic acid will lead to liberation of free intracellular carbonic acid and a slight increase in buffer base. The increased oxidations from super accumulation of lactic acid would contribute a final acid effect of increased free carbonic acid. Whether the increased oxidations are due to increased cellular lactic acid, to decreased cellular hydrogen ion concentration, to decreased hydrogen ion concentration at the cell membrane, or to other causes remains to

be learned. The relatively even distribution of bicarbonate in the body as indicated by the late blood samples, however, suggests that the tissues eventually turn more alkaline than normal even though they initially appear to turn more acid than normal.

## SUMMARY

The effects of intravenous injection of sodium bicarbonate were studied during administration of constant pulmonary ventilation.

The results of earlier experiments on gaseous exchange were confirmed. There was an increase in carbon dioxide elimination and an increase in oxygen consumption. The increase in carbon dioxide elimination was out of proportion to the increase in oxygen consumption leading to a large but temporary increase in the expiratory quotient.

Intravenous injection of bicarbonate produced a sudden decrease in hydrogen ion concentration of the blood which was followed by a slow return to normal values. The changes in the carbon dioxide capacity of the blood were closely related to the pH changes.

The results of Macleod and others on the appearance of lactic acid in the blood were confirmed. With injection there is a rapid accumulation of lactic acid soon followed by a tendency to return to preinjection values.

The carotid and femoral flow of blood was markedly increased.

The elimination of non-oxidative carbon dioxide was found to exceed the carbon dioxide equivalent of the net accumulation of lactic acid. This was taken to suggest that higher concentrations of bicarbonate led to a more exclusive reaction between bicarbonate and the lactic acid which was formed. But since the ratio of non-oxidative carbon dioxide to the carbon dioxide equivalent of lactic acid exceeded unity it was concluded that other factors leading to increased carbon dioxide elimination were at work. These factors may be the formation of other fixed acids, the increased volume flow of blood, and the increased carbon dioxide pressure produced by the addition of bicarbonate to a bicarbonate buffer mixture.

An outstanding result of intravenous injection of bicarbonate was the tremendous increase in the dissipation of energy.

Mean blood pressure and pulmonary ventilation were at their highest at the highest elimination of carbon dioxide which supports the view of a common mechanism of control.

Acid base exchange between blood and tissues is discussed. Whether the injection of bicarbonate leads to more than temporary increase in cellular acidity is left for another type of experimental analysis. The results, however, indicate that the late depression of respiratory movements is related to a late increase in the alkalinity of the tissues.

## BIBLIOGRAPHY

GESELL. 1923. Proc. Soc. Exper. Biol. and Med., xx, 345.
GESELL AND HERTZMAN. 1926. This Journal, lxxviii, 610.
GESELL AND MCGINTY. 1927. This Journal, lxxxiii, 445.
MACLEOD AND HOOVER. 1917. This Journal, xlii, 460.
MACLEOD. 1918. This Journal, xlv, 539.
MACLEOD AND KNAPP. 1918. This Journal, xlvii, 189.
MACLEOD. 1921. This Journal, lv, 184.
ANREP AND CANNON. 1923. Journ. Physiol., lviii, 244.
GESELL. 1928. Journ. Amer. Med. Assoc., xci, 1256.
1929. Ergebn. d. Physiol., 28.

# THE REGULATION OF RESPIRATION

## A Study of the Correlation of Numerous Factors of Respiratory Control Following Administration of Hydrochloric Acid, of Carbon Dioxide, and the Simultaneous Administration of Carbon Dioxide and Sodium Bicarbonate[1, 2]

ROBERT GESELL, HUGO KRUEGER,[3] GRACE GORHAM AND THEODORE BERNTHAL

*From the Department of Physiology, University of Michigan, Ann Arbor*

Received for publication March 17, 1930

In the preceding paper we studied effects of intravenous injection of sodium bicarbonate. We now describe effects of intravenous injection of hydrochloric acid and administration of carbon dioxide in inspired air under similar experimental conditions.

Results. The results of two injections are shown in experiment 1. The first injection of 225 cc. M/6 hydrochloric acid is equivalent to 38.45 cc. of carbon dioxide per kilogram body weight and the second injection of 325 cc. is equivalent to 55.5 cc. These equivalents are represented by the rectangular areas in the lower half of the graph. Preceding the first injection of hydrochloric acid it will be noticed that the hydrogen ion concentration, the carbon dioxide capacity and the lactic acid content of the blood are decreasing along with decreasing output of carbon dioxide. These changes are attributed to the greater effectiveness of the artificial ventilation as compared with the preceding normallly controlled ventilation with the chest intact. For a clearer analysis of the results it is, therefore, desirable to extend the preinjection gradients.

During the first injection, the hydrogen ion concentration of the blood increases. Blood carbon dioxide capacity and the blood lactic acid decrease. This decrease in blood lactic acid agrees with that noted by Anrep and Cannon on administration of carbon dioxide (1923–24). Oxygen consumption and energy production decrease and carbon dioxide

[1] Preliminary report, This Journal, 1929, xc, 360.

[2] This investigation has been made with the assistance of a grant from the Committee on Therapeutic Research, Council on Pharmacy and Chemistry, American Medical Association.

[3] Submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

elimination increases. As a result of increased elimination of non-oxidative carbon dioxide, plotted in the lower half of the graph, the expiratory quotient rises from 0.86 to 1.27.

During recovery, which begins at the end of the injection, there is first a relatively rapid decrease in the hydrogen ion concentration of the blood and then a more gradual decrease. Even so the blood becomes more alkaline than the initial value of pH 7.23. But it is more acid than the value immediately preceding injection. Thus if the preinjection gradient is extended the acid effect of hydrochloric acid is obvious. The changes

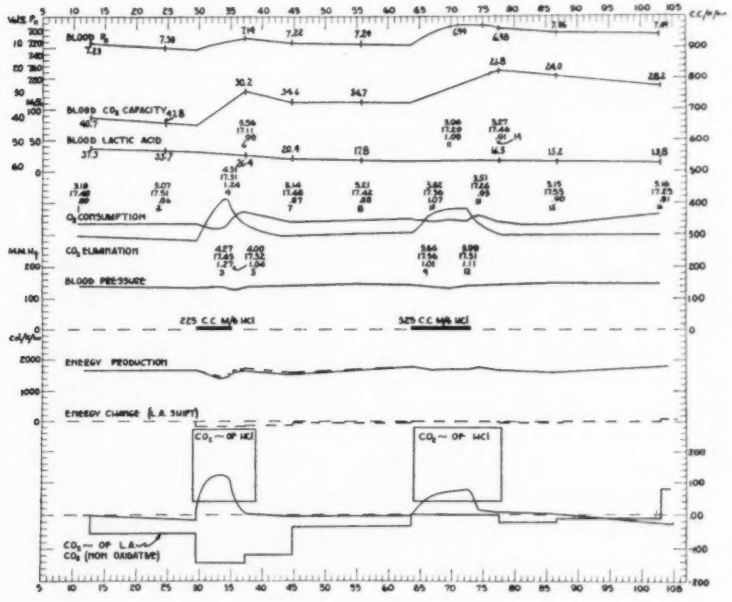



Fig. 1. Experiment 1

in blood carbon dioxide capacity are similar to the changes in hydrogen ion concentration, a rapid increase followed by a relatively constant value. Oxygen consumption increases and total and non-oxidative carbon dioxide elimination diminish. Oxygen consumption not only increases but overshoots the normal preadministration level. Then it slowly decreases but never quite reaches the preadministration level. Carbon dioxide elimination also remains above the preadministration level.

On the whole the effects of the second injection are very similar to those of the first injection. The blood becomes more acid and remains at a

TABLE 1

*Experiment 1. Hydrochloric acid*

| OBSERVATION | PROCEDURE | TIME | PER CENT OF EXPIRED $CO_2$ | PER CENT OF EXPIRED $O_2$ | EXPIRATORY QUOTIENT | $O_2$ CONSUMPTION IN CC. PER KGM. HR. | $CO_2$ ELIMINATION IN CC. PER KGM. HR. | $CO_2$ ELIMINATION FROM OXIDATIONS BASED ON E.Q. IN CC. PER KGM. HR. | DIFFERENCE BETWEEN ACTUAL AND THEORETICAL $CO_2$ ELIMINATION | $CO_2$ EQUIVALENT OF CHANGE OF LACTIC ACID | LACTIC ACID IN MGM. PER 100 CC. ARTERIAL BLOOD | $CO_2$ CAPACITY OF ARTERIAL BLOOD IN VOLUME PER CENT | pH OF ARTERIAL BLOOD | MEAN BLOOD PRESSURE | OXIDATIVE ENERGY IN SMALL CALORIES PER KGM. HR. | NON-OXIDATIVE ENERGY IN SMALL CALORIES PER KGM. HR. | OXIDATIVE + NON-OXIDATIVE ENERGY IN SMALL CALORIES PER KGM. HR. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 |
| 1 | G. S. no. 1 | 11′ 40″ | 3.18 | 17.48 | 0.89 | 335.4 | 297.2 | 297.2 | | | | | | 140 | 1,647 | | |
| 2 | A. S. no. 1 | 12′ 38″ | | | | | | | | | 37.3 | 40.72 | 7.23 | | | | |
| 3 | G. S. no. 2 | 23′ 35″ | 3.07 | 17.51 | 0.86 | 334.4 | 286.7 | 296.2 | −10 | −45 | | | | 136 | 1,642 | | |
| 4 | A. S. no. 2 | 24′ 33″ | | | | | | | | | 33.7 | 42.82 | 7.30 | | | | |
| 5 | N/6 HCl 225 cc. | 29′ 30″ | | | | | | | | | | | | | | | |
| 6 | G. S. no. 3 | 33′ 25″ | 4.27 | 17.43 | 1.27 | 318.6 | 405.0 | 282.2 | +123 | −144 | | | | 136 | 1,564 | −175 | 1,389 |
| 7 | G. S. no. 4 | 34′ 30″ | 4.31 | 17.31 | 1.24 | 331.0 | 409.2 | 293.2 | 116 | −144 | | | | 132 | 1,625 | −175 | 1,450 |
| 8 | G. S. no. 5 | 35′ 23″ | 4.00 | 17.32 | 1.04 | 358.1 | 372.0 | 317.2 | 55 | −144 | | | | 130 | 1,759 | −175 | 1,584 |
| 9 | G. S. no. 6 | 37′ 5″ | 3.56 | 17.11 | 0.90 | 369.9 | 333.2 | 327.8 | 6 | −144 | | | | 134 | 1,815 | −175 | 1,640 |
| 10 | A. S. no. 3 | 37′ 18″ | | | | | | | | | 26.4 | 30.22 | 7.14 | | | | |
| 11 | G. S. no. 7 | 44′ 25″ | 3.14 | 17.48 | 0.87 | 339 | 296.7 | 300.5 | −4 | −119 | | | | 142 | 1,665 | −145 | 1,520 |
| 12 | A. S. no. 4 | 44′ 51″ | | | | | | | | | 20.4 | 34.55 | 7.22 | | | | |
| 13 | G. S. no. 8 | 55′ 28″ | 3.21 | 17.42 | 0.88 | 346.1 | 302.9 | 306.8 | −4 | −36 | | | | 146 | 1,700 | −43 | 1,657 |
| 14 | A. S. no. 5 | 55′ 48″ | | | | | | | | | 17.8 | 34.68 | 7.24 | | | | |
| 15 | m/6 HCl 325 cc. | 63′ 38″ | | | | | | | | | c 16.5 | | | 143 | | | |
| 16 | G. S. no. 9 | 65′ 55″ | 3.66 | 17.36 | 1.01 | 342.8 | 347.5 | 303.8 | +44 | 0 | | | | | 1,682 | | |
| 17 | G. S. no. 10 | 66′ 50″ | 3.82 | 17.36 | 1.07 | 340.2 | 364.2 | 301.5 | 63 | 0 | | | | | 1,670 | | |
| 18 | G. S. no. 11 | 69′ 39″ | 3.96 | 17.29 | 1.09 | 345.1 | 377.4 | 306 | 71 | 0 | | | | 132 | 1,695 | | |
| 19 | A. S. no. 5A | 71′ 4″ | | | | | | | | | | | 6.94 | | | | |

| | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 20 | G. S. no. 12 | 72′ 34″ | 3.99 | 17.31 | 1.11 | 342.2 | 381.0 | 303.3 | 78 | 0 | | | | 140 | 1,680 | | |
| 21 | G. S. no. 13 | 74′ 18″ | 3.51 | 17.26 | 0.93 | 358 | 332.3 | 317.2 | 15 | 0 | | | | | 1,758 | | |
| 22 | G. S. no. 14 | 77′ 8″ | 3.27 | 17.46 | 0.91 | 339 | 309.3 | 300.4 | 9 | 0 | | | | | 1,665 | | |
| 23 | A. S. no. 6 | 77′ 33″ | | | | | | | | | 16.5 | 21.76 | 6.98 | | | | |
| 24 | G. S. no. 15 | 85′ 53″ | 3.15 | 17.55 | 0.90 | 330.3 | 296.9 | 292.8 | 4 | −21 | | | | 148 | 1,622 | −26 | 1,596 |
| 25 | A. S. no. 7 | 86′ 37″ | | | | | | | | | 15.2 | 24.04 | 7.06 | | | | |
| 26 | G. S. no. 16 | 103′ | 3.16 | 17.25 | 0.81 | 364.9 | 296.9 | 323.2 | −26 | −12 | | | | 148 | 1,790 | −15 | 1,775 |
| 27 | A. S. no. 8 | 103′ | | | | | | | | | 13.8 | 28.24 | 7.09 | | | | |
| | Stopped drum, changed paper | 103′ | | | | | | | | | | | | | | | |
| 28 | G. S. no. 17 | 107′ 18″ | 2.99 | 17.40 | 0.80 | 351.6 | 280.6 | 311.8 | −31 | +81 | | | | 153 | 1,725 | +97 | 1,822 |
| 29 | A. S. no. 9 | 107′ 50″ | | | | | | | | | 16.4 | 27.78 | 7.12 | | | | |
| 30 | mNa $HCO_3$ 250 cc. | 110′ 35″ | | | | | | | | | c 18.7 | | | 154 | | | |
| 31 | G. S. no. 18 | 113′ 25″ | 6.49 | 15.55 | 1.26 | 495.6 | 625.4 | 429 | 196 | +386 | | | | 176 | 2,431 | +469 | 2,900 |
| 32 | A. S. no. 10 | 115′ 48″ | | | | | | | | | 31.4 | 93.23 | 7.56 | 163 | | | |
| 33 | A. S. no. 11 | 119′ 38″ | | | | | | | | | 29.9 | 81.01 | 7.55 | 158 | | | |
| 34 | A. S. no. 12 | 134′ 57″ | | | | | | | | | 31.7 | 68.49 | 7.51 | 165 | | | |
| 35 | G. S. no. 19 | 136′ 57″ | 4.04 | 16.81 | 0.96 | 398.0 | 382.5 | 353 | +29 | +18 | | | | | 1,954 | +21 | 1,975 |
| 36 | G. S no. 20 | 155′ 5″ | 3.88 | 16.89 | 0.93 | 392.3 | 366.4 | 348 | +18 | +22 | | | | 165 | 1,922 | +27 | 1,949 |
| 37 | A. S. no. 13 | 155′ 20″ | | | | | | | | | 34.7 | 61.90 | 7.50 | | | | |

higher acid level. Blood carbon dioxide capacity is reduced still more but shows a greater tendency to return to the preinjection level. Blood lactic acid changes are very small. Oxygen consumption diminishes during injection and increases during recovery. The overshooting is less than in the preceding injection. Non-oxidative carbon dioxide increases, but not as much as with the first injection. Energy production changes are in the same direction but of a smaller order.

Comparison of the areas of non-oxidative carbon dioxide with the corresponding areas of the carbon dioxide equivalents of the hydrochloric acid injected indicates the proportion of hydrochloric acid reacting with bicarbonate. In the first injection the non-oxidative carbon dioxide between gas samples 1 and 8 is 6.89 cc. per kgm. which is equal to 18 per cent of the carbon dioxide equivalent of the hydrochloric acid injected. From the second injection up to gas sample 16 the non-oxidative carbon dioxide is equal to 11.22 cc. per kgm. which is 20 per cent of the carbon dioxide equivalent of the second acid injection. In these determinations the effects of the lactic acid shift on absorption and liberation of carbon dioxide are not considered. To be sure the nature of this shift is not understood but assuming an equal disappearance of lactic acid in the blood and tissues and an equivalent formation of bicarbonate we find that with the first injection non-oxidative carbon dioxide is equal to twice the carbon dioxide equivalent of the hydrochloric acid injected. (Carbon dioxide equivalent of acid injected = 38.45 cc. per kgm. Carbon dioxide equivalent of lactic acid disappearing = 35 cc. per kgm. Non-oxidative carbon dioxide = 6.89 cc. per kgm. Therefore, 6.89 cc. ÷ (38.45 cc. − 35.00 cc.) = 2.00). Obviously the assumption is wrong. Perhaps lactic acid changes are of a different order or of opposite direction in blood and tissue. This remains to be determined by direct experiment. With the second injection the lactic acid changes are much less significant.

By comparing the actual decrease in the blood carbon dioxide capacity resulting from injection of hydrochloric acid with the expected decrease, assuming equal distribution of hydrochloric acid between blood and tissues and similar reaction with bicarbonate and other buffers in the blood and tissues, it is found that the actual decrease is greater than the expected decrease. The tentative conclusion is that the distribution of acid is uneven. For example, with the first injection assuming an 18 per cent reaction between hydrochloric acid and bicarbonate throughout the body a 0.69 volume per cent decrease in carbon dioxide capacity of the blood is to be expected. The actual decrease obtained is 9.00 volumes per cent indicating that the concentration of hydrochloric acid appears to be 13 times as great in the blood as in the tissues. With the second injection the concentration appears to be 5 times as great as in the tissues. It is of course realized that these computations may be subject to gross error,

but our results agree with the ammonium chloride experiments of Haldane (1921) in which he shows a greater concentration of the liberated hydrochloric acid in the blood than in the tissues.

In experiment 2 there were three injections. The carbon dioxide equivalent of the first injection of hydrochloric acid was 45.6 cc. per kgm., of the following injection of sodium bicarbonate 242. cc. per kgm. and of the second injection of hydrochloric acid 21.2 cc. per kgm.

The beneficial effects of artificial ventilation are seen again during the preinjection period. The initial pH of the blood of this experiment is about 0.2 pH more alkaline at the outset than in experiment 1. Yet the effects of acid injection are more severe. Oxygen consumption is diminished not only during injection but during the subsequent period as well. There is a short period of recovery which gives way to a rapid failure which is seen in the rate of oxygen consumption, in the diminution of carbon dioxide elimination, the fall of mean blood pressure and the rise in blood lactic acid. The effects of this injection are of particular interest in showing both a fall and a rise in blood lactic acid with decreasing oxidations. There is the usual increase in the expiratory quotient and the tendency towards hyperoxidations is seen in the hump on the curve of oxygen consumption.

The rapid collapse of the animal is suddenly reversed by injection of sodium bicarbonate. The usual effects of intravenous injection of bicarbonate are seen. It is thus interesting to note the increasing lactic acid content of the blood which is associated with impaired oxidations after injection of hydrochloric acid suddenly augmented during a period of superoxidations. Apparently rate of oxidations is not the only factor determining lactic acid accumlation. Oxidations and mean blood pressure are well maintained. Lactic acid content of the blood reaches a fairly constant but higher level than before the injection of hydrochloric acid. This is associated with a relatively high pH.

Though the blood is very alkaline and has a high carbon dioxide capacity the second and smaller injection of hydrochloric acid has far more serious effects. The circulatory failure is very abrupt. The severe reduction of oxidations is accompanied by a very sharp increase in blood lactic acid.

The ratio of non-oxidative carbon dioxide eliminated from the beginning of injection of hydrochloric acid to gas sample 5, which is 19.48 cc. per kgm., to the carbon dioxide equivalent of the acid injected (45.6 cc.) indicates that 23 per cent of the hydrochloric acid reacts with bicarbonate. On the other hand, if it is assumed that the disappearance of lactic acid yields equivalent base for reaction with carbon doxide the ratio becomes 19.48 to 37.35 indicating that 52 per cent of the hydrochloric acid reacts with bicarbonate. For the more inclusive period from the injection of hydrochloric acid to the injection of sodium bicarbonate there is no difference

TABLE 2

*Experiment 2. Hydrochloric acid*

| OBSERVATION | PROCEDURE | TIME | PER CENT OF EXPIRED $CO_2$ | PER CENT OF EXPIRED $O_2$ | EXPIRATORY QUOTIENT | $O_2$ CONSUMPTION IN CC. PER KGM. HR. | $CO_2$ ELIMINATION IN CC. PER KGM. HR. | $CO_2$ ELIMINATION FROM OXIDATIONS BASED ON E.Q. IN CC. PER KGM. HR. | DIFFERENCE BETWEEN ACTUAL AND THEORETICAL $CO_2$ ELIMINATION | $CO_2$ EQUIVALENT OF CHANGE OF LACTIC ACID | LACTIC ACID IN MGM. PER 100 CC. ARTERIAL BLOOD | $CO_2$ CAPACITY OF ARTERIAL BLOOD IN VOLUME PER CENT | pH OF ARTERIAL BLOOD | MEAN BLOOD PRESSURE | OXIDATIVE ENERGY IN SMALL CALORIES PER KGM. HR. | NON-OXIDATIVE ENERGY IN SMALL CALORIES PER KGM. HR. | OXIDATIVE + NON-OXIDATIVE ENERGY IN SMALL CALORIES PER KGM. HR. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 |
| 1 | G. S. no. 1 | 8′ 48″ | 2.91 | 17.67 | 0.85 | 383.7 | 325.8 | 325.8 | | | | | | 130 | 1,866 | | |
| 2 | A. S. no. 1 | 9′ 28″ | | | | | | | | | 36.8 | 57.60 | 7.52 | | | | |
| 3 | G. S. no. 2 | 19′ 20″ | 2.88 | 17.67 | 0.84 | 384.5 | 322.3 | 326.3 | −4 | −59 | | | | 119 | 1,869 | | |
| 4 | A. S. no. 2 | 20′ 5″ | | | | | | | | | 32.9 | 57.00 | 7.57 | | | | |
| 5 | m/HCl 215 cc. | 22′ 28″ | | | | | | | | | c 32.1 | | | 111 | | | |
| 6 | G. S. no. 3 | 28′ 2″ | 3.99 | 17.76 | 1.33 | 342.3 | 455.6 | 290.7 | 165 | −43 | | | | 111 | 1,664 | −52 | 1,612 |
| 7 | G. S. no. 4 | 30′ 11″ | 4.13 | 17.63 | 1.32 | 357.3 | 471.5 | 303.3 | 168 | −43 | | | | 100 | 1,738 | −52 | 1,686 |
| 8 | A. S. no. 2A | 30′ 22″ | | | | | | | | | | | 7.14 | | | | |
| 9 | G. S. no. 5 | 33′ 45″ | 2.71 | 18.32 | 1.02 | 297.9 | 304.4 | 253 | 51 | −43 | | | | 95 | 1,448 | −52 | 1,396 |
| 10 | A. S. no. 3 | 34′ 18″ | | | | | | | | | 28.7 | 36.20 | 7.34 | | | | |
| 11 | G. S. no. 6 | 41′ 32″ | 1.96 | 18.99 | 0.98 | 223.9 | 218.5 | 190 | 29 | +42 | | | | 62 | 1,088 | +51 | 1,139 |
| 12 | A. S. no. 4 | 42′ 12″ | | | | | | | | | 30.9 | 38.49 | 7.34 | | | | |
| 13 | $mNaHCO_3$ 190 cc. | 46′ 41″ | | | | | | | | | c 32.1 | | | 56 | | | |
| 14 | G. S. no. 7 | 48′ 30″ | 1.73 | 20.15 | 3.06 | 65.6 | 200.7 | 55.8 | 145 | +708 | | | | 36 | 319 | +855 | 1,163 |
| 15 | G. S. no. 8 | 50′ 18″ | 6.61 | 15.95 | 1.44 | 546.5 | 786.7 | 464.2 | 322 | +708 | | | | | 2,658 | +855 | 3,513 |
| 16 | G. S. no. 9 | 51′ 1″ | 7.15 | 15.49 | 1.42 | 600.6 | 851.4 | 510 | 341 | +708 | | | | | 2,920 | +855 | 3,777 |
| 17 | G. S. no. 10 | 51′ 55″ | 7.36 | 15.70 | 1.56 | 564.6 | 882.1 | 480 | 402 | +708 | | | | | 2,748 | +855 | 3,603 |
| 18 | G. S. no. 11 | 52′ 42″ | 6.95 | 16.05 | 1.59 | 524.1 | 831.4 | 445 | 386 | +708 | | | | 85 | 2,550 | +855 | 3,405 |

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 19 | G. S. no. 12 | 54′ 37″ | 5.60 | 17.13 | 1.66 | 401.9 | 666.8 | 341 | 326 | +708 | | | | 75 | 1,953 | +855 | 2,808 |
| 20 | A. S. no. 5 | 54′ 45″ | | | | | | | | | 70.1 | 95.49 | 7.69 | 88 | 1,836 | −9 | 1,827 |
| 21 | G. S. no. 13 | 64′ 12″ | 3.45 | 17.72 | 1.08 | 377.6 | 406.4 | 320.7 | 86 | −8 | | | | | | | |
| 22 | A. S. no. 6 | 64′ 45″ | | | | | | | | | 69.6 | 77.65 | 7.70 | 85 | 1,655 | +28 | 1,683 |
| 23 | G. S. no. 14 | 78′ 12″ | 3.07 | 18.05 | 1.06 | 340.4 | 361.3 | 289 | 72 | +23 | | | | | | | |
| 24 | A. S. no. 7 | 78′ 59″ | | | | | | | | | 71.9 | 67.78 | 7.66 | 80 | 1,494 | +154 | 1,648 |
| 25 | G. S. no. 15 | 86′ 13″ | 2.91 | 18.30 | 1.11 | 308.2 | 341.9 | 261.8 | 80 | +127 | | | | | | | |
| 26 | A. S. no. 8 | 86′ 54″ | | | | | | | | | 78.6 | 66.67 | 7.65 | 75 | | | |
| 27 | m/6HCl 100 cc. | 89′ 1″ | | | | | | | | | c 80.3 | | | 75 | 1,491 | +258 | 1,749 |
| 28 | G. S. no. 16 | 91′ 33″ | 4.36 | 18.03 | 1.71 | 307.0 | 523.9 | 260.7 | 263 | +212 | | | | | | | |
| 29 | A. S. no. 8A | 92′ 3″ | | | | | | | | | | | 7.38 | 66 | 1,388 | +258 | 1,646 |
| 30 | G. S. no. 17 | 93′ 1″ | 3.18 | 18.39 | 1.31 | 287.4 | 376.5 | 244 | 132 | +212 | | | | 64 | 1,204 | +258 | 1,462 |
| 31 | G. S. no. 18 | 95′ 35″ | 2.69 | 18.75 | 1.28 | 247.1 | 316.6 | 210 | 107 | +212 | | | | | | | |
| 32 | A. S. no. 9 | 96′ 23″ | | | | | | | | | 90.7 | 53.03 | 7.52 | 33 | 248 | +626 | 894 |
| 33 | G. S. no. 19 | 102′ 43″ | 0.81 | 20.44 | 1.80 | 51.1 | 91.8 | 43.4 | 48 | +515 | | | | | | | |
| 34 | A. S. no. 10 | 102′ 43″ | | | | | | | | | 112.4 | 52.59 | No pH | | 689 | +1,038 | 1,727 |
| 35 | G. S. no. 20 | 106′ 57″ | 1.70 | 19.66 | 1.40 | 141.6 | 198.3 | 120.2 | 78 | +852 | | | | | | | |
| 36 | A. S. no. 11 | 107′ 27″ | | | | | | | | | 139.3 | 48.86 | No pH | | | | |

in lactic acid content of the blood. The corresponding non-oxidative carbon dioxide is 29.04 cc. which gives a 64 per cent effectiveness of hydrochloric acid in driving off carbon dioxide.

From the injection of sodium bicarbonate to the injection of hydrochloric acid 87.75 cc. per kgm. of non-oxidative carbon dioxide is eliminated. The source of this carbon dioxide has been discussed in the preceding

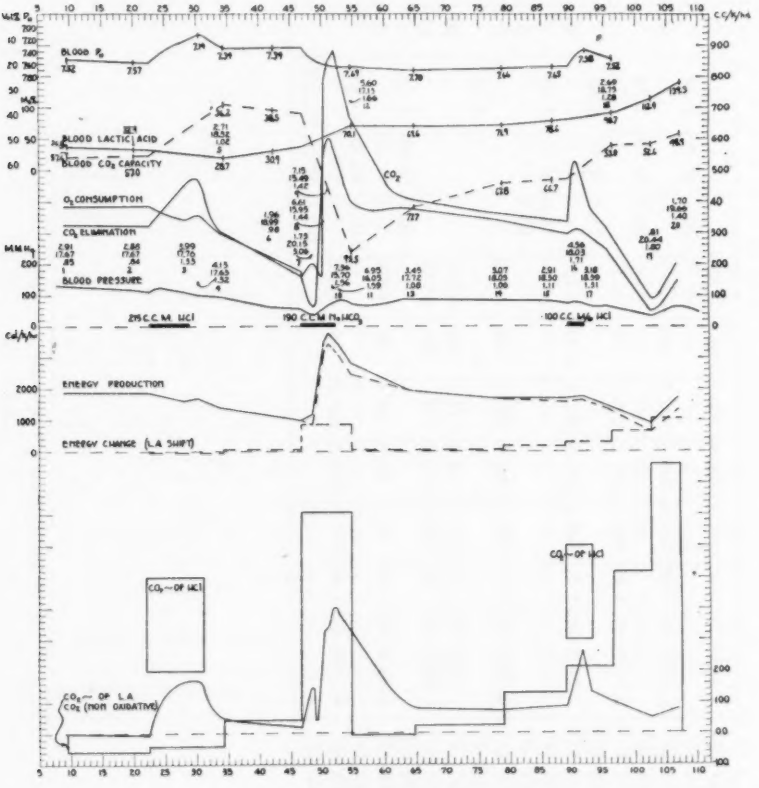



Fig. 2. Experiment 2

paper. It suffices to say that this is equal to 36 per cent of the carbon dioxide equivalent of the injected bicarbonate. The amount of lactic acid formed as a result of administration of bicarbonate is 48.2 mgm. per kgm. or the equivalent of 120 5 cc. The lactic acid formed is, therefore, equivalent to 52 per cent of the bicarbonate injected.

With the last injection of hydrochloric acid it is impossible to arrive even

at an approximate ratio of the reaction of the hydrochloric acid with bicarbonate due to the rapid formation of lactic acid which increases the non-oxidative carbon dioxide. The non-oxidative carbon dioxide for the period following injection up to gas sample 20 is 30 cc. which is greater than the carbon dioxide equivalent of the acid injection, namely, 21.2 cc.

The amount of hydrochloric acid leaving the blood stream is again very small according to the method of computation employed. Assuming that 64 per cent of the hydrochloric acid reacts with bicarbonate (this is a high figure) there should be a reduction of 2.92 volumes per cent in the carbon dioxide capacity of the blood with the first injection of acid. The actual reduction is 17.65 volumes per cent indicating that 6 times as much acid remained in the blood as might be expected with uniform distribution in the body.

This may be compared with the movement of bicarbonate from the blood stream following bicarbonate injection. The increase in carbon dioxide capacity expected from injection on the basis of uniform distribution is 24.2 volumes per cent and that obtained is 26.50. When the neutralizing effects of lactic acid and the excretion of bicarbonate of the kidneys is considered, however, the difference would increase. Yet it appears that the movement of base from the blood is freer than the movement of hydrochloric acid.

By adding the $CO_2$ equivalent of the injected bicarbonate 24.2 volumes per cent to the original carbon dioxide capacity of the blood 57.0 volumes per cent we get a total of 81.2 volumes per cent. At gas sample 20 the carbon dioxide capacity of the blood is 48.25 volumes per cent indicating a disappearance of 32.95 volumes per cent. It is interesting to note that the $CO_2$ equivalent of the two injections of hydrochloric acid and of the lactic acid formed is very nearly the same. (First injection of HCl $-$ 45.6 volumes per cent plus second injection of hydrochloric acid $-$ 2.12 volumes per cent plus lactic acid increase $-$ 26.05 volumes per cent = 32.73 volumes per cent.) This does not of course mean that there has been complete reaction between bicarbonate and acid. Undoubtedly a considerable amount of base has been excreted by the kidneys. At least that would be in agreement our other figures on the ratio of non-oxidative carbon dioxide to the carbon dioxide equivalent of the acid concerned.

In experiment 3 effects of four injections are shown, two of hydrochloric acid, one of sodium bicarbonate and one of isotonic saline solution. The carbon dioxide equivalent of each of the injections of hydrochloric acid is 3.61 volumes per cent and of the bicarbonate injection 14.45 volumes per cent.

The usual effects of acid injection on the hydrogen ion concentration of the blood and on the carbon dioxide capacity of the blood are evident. The initial effect of the first injection of hydrochloric acid is a reduction

TABLE 3

*Experiment 3. Hydrochloric acid*

| OBSERVATION | PROCEDURE | TIME | PER CENT OF EXPIRED $CO_2$ | PER CENT OF EXPIRED $O_2$ | EXPIRATORY QUOTIENT | $O_2$ CONSUMPTION IN CC. PER KGM. HR. | $CO_2$ ELIMINATION IN CC. PER KGM. HR. | $CO_2$ ELIMINATION FROM OXIDATIONS BASED ON E.Q. IN CC. PER KGM. HR. | DIFFERENCE BETWEEN ACTUAL AND THEORETICAL $CO_2$ ELIMINATION | $CO_2$ EQUIVALENT OF CHANGE OF LACTIC ACID | LACTIC ACID IN MGM. PER 100 CC. ARTERIAL BLOOD | $CO_2$ CAPACITY OF ARTERIAL BLOOD IN VOLUME PER CENT | pH OF ARTERIAL BLOOD | MEAN BLOOD PRESSURE | OXIDATIVE ENERGY IN SMALL CALORIES PER KGM. HR. | NON-OXIDATIVE ENERGY IN SMALL CALORIES PER KGM. HR. | OXIDATIVE + NON-OXIDATIVE ENERGY IN SMALL CALORIES PER KGM. HR. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 |
| 1 | G. S. no. 1 | 1′ 58″ | 2.64 | 18.14 | 0.91 | 280 | 255.7 | 255.7 | | | | | | 110 | 1,384 | | |
| 2 | A. S. no. 1 | 2′ 53″ | | | | | | | | | 40.8 | 37.27 | 7.22 | | | | |
| 3 | G. S. no. 2 | 11′ 56″ | 2.61 | 18.20 | 0.93 | 273.6 | 253 | 245 | 8 | | | | | 98 | 1,352 | | |
| 4 | A. S. no. 2 | 12′ 46″ | | | | | | | | | 41.6 | 40.66 | 7.26 | | | | |
| 5 | m/6 HCl 300 cc. | 16′ 42″ | | | | | | | | | c 42 | | | 85 | | | |
| 6 | G. S. no. 3 | 18′ 35″ | 3.01 | 18.24 | 1.13 | 260.4 | 293.4 | 237.8 | 56 | −74 | | | | | 1,286 | −90 | 1,196 |
| 7 | G. S. no. 4 | 20′ 6″ | 3.08 | 18.20 | 1.14 | 262.8 | 300.5 | 240 | 61 | −74 | | | | 86 | 1,290 | −90 | 1,200 |
| 8 | G. S. no. 5 | 22′ 42″ | 3.15 | 18.24 | 1.20 | 256.7 | 307.6 | 234.4 | 73 | −74 | | | | | 1,268 | −90 | 1,178 |
| 9 | A. S. no. 2A | 24′ 57″ | | | | | | | | | | | 6.94 | | | | |
| 10 | G. S. no. 6 | 25′ 36″ | 3.02 | 18.29 | 1.16 | 253.6 | 294.8 | 231.6 | 63 | −74 | | | | 86 | 1,254 | −90 | 1,164 |
| 11 | G. S. no. 7 | 27′ 32″ | 2.73 | 18.31 | 1.03 | 258.4 | 265.1 | 236 | 29 | −74 | | | | | 1,272 | −90 | 1,182 |
| 12 | G. S. no. 8 | 29′ 58″ | 2.55 | 18.40 | 0.98 | 251.2 | 246.8 | 229.3 | 18 | −74 | | | | 80 | 1,240 | −90 | 1,150 |
| 13 | A. S. no. 3 | 30′ 8″ | | | | | | | | | 35.4 | 27.86 | 7.01 | | | | |
| 14 | G. S. no. 9 | 33′ 39″ | 2.35 | 18.56 | 0.96 | 236.3 | 227.2 | 215.8 | 12 | −36 | | | | 78 | 1,167 | −44 | 1,123 |
| 15 | G. S. no. 10 | 37′ 9″ | 2.34 | 18.56 | 0.96 | 236.2 | 226.8 | 215.7 | " | −36 | | | | 74 | 1,168 | −44 | 1,124 |
| 16 | A. S. no. 4 | 37′ 9″ | | | | | | | | | 33.7 | 27.79 | 7.07 | | | | |
| 17 | G. S. no. 11 | 45′ 31″ | 2.20 | 18.72 | 0.97 | 220.3 | 212.6 | 201.2 | " | +112 | | | | 66 | 1,090 | +136 | 1,226 |
| 18 | A. S. no. 5 | 46′ 22″ | | | | | | | | | 40.5 | 28.46 | 7.09 | | | | |
| 19 | G. S. no. 12 | 51′ 16″ | 2.20 | 18.78 | 1.00 | 212.9 | 212.6 | 194.4 | 18 | | | | | | 1,052 | +136 | 1,188 |

| | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 20 | Saline 400 cc. | 51′ 39″ | | | | | | | | | c 43.9 | | | 60 | | | |
| 21 | G. S. no. 13 | 53′ 16″ | 3.12 | 17.71 | 0.94 | 318.5 | 300.8 | 291 | 10 | −161 | | | | 80 | 1,574 | −196 | 1,378 |
| 22 | G. S. no. 14 | 54′ 20″ | 3.25 | 17.92 | 1.08 | 291.3 | 314.8 | 266 | 49 | −161 | | | | | 1,440 | −196 | 1,244 |
| 23 | G. S. no. 15 | 58′ 17″ | 2.75 | 18.38 | 1.08 | 246.6 | 265.4 | 225.2 | 40 | −161 | | | | 80 | 1,218 | −196 | 1,022 |
| 24 | A. S. no. 6 | 58′ 27″ | | | | | | | | | 36.6 | 26.33 | 7.05 | | | | |
| 25 | G. S. no. 16 | 67′ 14″ | 2.38 | 18.53 | 0.97 | 237.0 | 228.7 | 216.3 | 12 | −73 | | | | 74 | 1,171 | −89 | 1,082 |
| 26 | A. S. no. 7 | 67′ 28″ | | | | | | | | | 32.2 | 30.13 | 7.07 | | | | |
| 27 | m/Na $HCO_3$ 200 cc. | 69′ 53″ | | | | | | | | | 31.1 | | | 69 | | | |
| 28 | G. S. no. 17 | 71′ 5″ | 5.64 | 17.02 | 1.61 | 347.4 | 559.4 | 317.2 | 242 | +632 | | | | | 1,716 | +768 | 2,484 |
| 29 | G. S. no. 18 | 72′ 17″ | 5.67 | 17.22 | 1.75 | 322.0 | 563.8 | 294 | 270 | +632 | | | | 79 | 1,586 | +768 | 2,354 |
| 30 | G. S. no. 19 | 74′ 5″ | 5.04 | 17.44 | 1.61 | 309.3 | 498 | 282.4 | 216 | +632 | | | | | 1,528 | +768 | 2,296 |
| 31 | G. S. no. 20 | 76′ 1″ | 4.47 | 17.73 | 1.53 | 286.4 | 439.4 | 261.6 | 178 | +632 | | | | 90 | 1,415 | +768 | 2,183 |
| 32 | A. S. no. 8 | 76′ 1″ | | | | | | | | | 56.9 | 75.72 | 7.47 | | | | |
| 33 | G. S. no. 21 | 80′ 12″ | 3.58 | 17.94 | 1.24 | 281.0 | 347.9 | 256.6 | 91 | −11 | | | | 80 | 1,388 | −13 | 1,375 |
| 34 | A. S. no. 9 | 80′ 15″ | | | | | | | | | 56.6 | 66.45 | 7.48 | | | | |
| 35 | G. S. no. 22 | 90′ 47″ | 3.03 | 18.14 | 1.09 | 269.7 | 293.1 | 246.2 | 47 | −14 | | | | 70 | 1,333 | +6 | 1,339 |
| 36 | A. S. no. 10 | 90′ 45″ | | | | | | | | | 56.9 | 61.39 | 7.44 | | | | |
| 37 | m/6 HCl 300 cc. | 94′ 3″ | | | | | | | | | 57.0 | | | 70 | | | |
| 38 | G. S. no. 23 | 95′ 40″ | 3.15 | 18.09 | 1.11 | 267.7 | 298.1 | 244.3 | 54 | −111 | | | | 86 | 1,323 | −135 | 1,187 |
| 39 | G. S. no. 24 | 99′ 35″ | 4.14 | 17.83 | 1.44 | 275.6 | 397.2 | 251.7 | 146 | −111 | | | | 84 | 1,362 | −135 | 1,227 |
| 40 | A. S. no. 10A | 99′ 5″ | | | | | | | | | | | 7.16 | | | | |
| 41 | G. S. no. 25 | 104′ | 4.08 | 17.86 | 1.43 | 273.6 | 391.0 | 249.8 | 141 | −111 | | | | 76 | 1,352 | −135 | 1,217 |
| 42 | A. S. no. 11 | 104′ 18″ | | | | | | | | | 49.4 | 42.52 | 7.28 | | | | |
| 43 | G. S. no. 26 | 111′ | 2.78 | 18.28 | 1.04 | 253.0 | 262.7 | 231 | 32 | −21 | | | | | 1,250 | −26 | 1,224 |
| 44 | A. S. no. 12 | 111′ | | | | | | | | | 48.4 | 45.29 | 7.13 | | | | |

in the lactic acid content of the blood from approximately 42 mgm. per 100 cc. to 34 mgm. This is followed by an increase back to the initial value. The general improvement produced by saline injection is associated with a second drop to 32 mgm. This low value then rises to 57 mgm. following injection of sodium bicarbonate. The final injection of acid has the same effects of the first—an initial decrease in lactic acid followed by an increase. The effects of injection of acid and of base on carbon dioxide

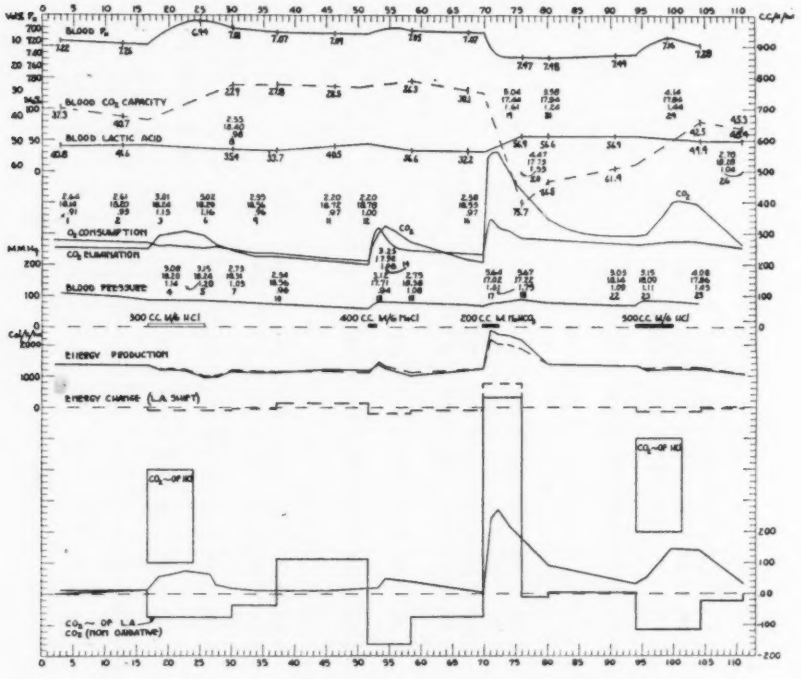



Fig. 3. Experiment 3

elimination are similar to those already described and are associated with the usual increase in the expiratory quotient. The effects of acid injection on oxygen consumption are somewhat different. While the first injection is associated with a decrease, the decrease is very small and with the second injection there is a slight increase. Even though the increased oxygen consumption may be explained by the augmentation of pulmonary ventilation the results suggest that in this experiment at least oxidations of the resting tissue were impaired very little by either injection of acid.

Assuming that hydrochloric acid is the only fixed acid combining with bicarbonate the reaction is 40 per cent complete for the period beginning with the injection of hydrochloric acid and ending with the injection of saline solution. (Non-oxidative carbon dioxide = 14.47 cc.; the carbon dioxide equivalent of the hydrochloric acid = 36.1 cc.) The completeness of reaction of the combined acids (hydrochloric acid 36.1 cc. plus the increase in lactic acid 4.75 cc.) is 35 per cent. The decrease in the carbon dioxide capacity resulting from the injection of acid is 13.25 volumes per cent which is over three times as great as the expected reduction (4.08 volumes per cent) assuming equal distribution of acid between the blood and tissues. From saline injection to the injection of bicarbonate there is an observed increase in blood carbon dioxide capacity of 2.25 volumes per cent accompanied by a decrease in the lactic acid content of the blood equivalent to 3.2 volumes per cent. Assuming that the disappearance of lactic acid gives rise to an equivalent formation of bicarbonate the expected decrease in carbon dioxide capacity of the blood from the injection of hydrochloric acid up to the moment just prior to injection of bicarbonate would be only 0.88 volume per cent. The observed decrease is 11 volumes per cent. A large proportion of the hydrochloric acid, therefore, appears to be in the blood.

From the injection of sodium bicarbonate up to the second injection of hydrochloric acid 45.99 cc. per kgm. of non-oxidative carbon dioxide are eliminated. This is equal to 32 per cent of the carbon dioxide equivalent of the bicarbonate injected (144.5 cc. per kgm.). The lactic acid produced as a result of the injection is equivalent to 64.75 cc. per kgm. of 45 per cent of the carbon dioxide equivalent of the bicarbonate. The carbon dioxide capacity of the blood before administration of bicarbonate is 31 volumes per cent and after administration 59.75 volumes per cent giving an increase of 18.75 volumes per cent. The expected increase was 14.45 volumes per cent. The effectiveness of lactic acid to combine with bicarbonate as represented by the ratio of the increase in lactic acid in terms of carbon dioxide equivalent (64.75 cc.) to the non-oxidative carbon dioxide (45.99 cc.) equals 144 per cent. A partial explanation of this high ratio has been attempted in the preceding paper.

The period of observation following the second injection is rather short for purposes of computation but if we are willing to risk a comparison it appears that the percentage of hydrochloric acid combining with bicarbonate after bicarbonate injection is greater than before. With the first injection the carbon dioxide index was 0.35 and with the second injection it was 0.79. The amount of bicarbonate present apparently determines how much will compete with the other buffers for acid. This factor presumably contributes to the high carbon dioxide index of lactic acid which is produced by the injection of sodium bicarbonate.

TABLE 4

*Experiment 4. Hydrochloric acid*

| OBSERVATION | PROCEDURE | TIME | PER CENT OF EXPIRED $CO_2$ | PER CENT OF EXPIRED $O_2$ | EXPIRATORY QUOTIENT | $O_2$ CONSUMPTION IN CC. PER KGM. HR. | $CO_2$ ELIMINATION IN CC. PER KGM. HR. | $CO_2$ ELIMINATION FROM OXIDATIONS BASED ON E.Q. IN CC. PER KGM. HR. | DIFFERENCE BETWEEN ACTUAL AND THEORETICAL $CO_2$ ELIMINATION | $CO_2$ EQUIVALENT OF CHANGE OF LACTIC ACID | LACTIC ACID IN MGM. PER 100 CC. ARTERIAL BLOOD | $CO_2$ CAPACITY OF ARTERIAL BLOOD IN VOLUME PER CENT | pH OF ARTERIAL BLOOD | MEAN BLOOD PRESSURE | OXIDATIVE ENERGY IN SMALL CALORIES PER KGM. HR. | NON-OXIDATIVE ENERGY IN SMALL CALORIES PER KGM. HR. | OXIDATIVE + NON-OXIDATIVE ENERGY IN SMALL CALORIES PER KGM. HR. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 |
| 1 | G. S. no. 1 | 34″ | 2.15 | 18.75 | 0.95 | 354.4 | 338.2 | | | | | | | 120 | | | |
| 2 | A. S. no. 1 | 1′ 50″ | | | | | | | | | 25.0 | 43.12 | 7.26 | | | | |
| 3 | G. S. no. 2 | 15′ 40″ | 2.17 | 18.40 | 0.80 | 422.5 | 339.5 | 339.5 | | +10 | | | | | 2,032 | +12 | 2,044 |
| 4 | A. S. no. 2 | 16′ 18″ | | | | | | | | | 25.9 | 41.54 | 7.21 | 114 | | | |
| 5 | M $NaHCO_3$ 200 cc. | 18′ 3″ | | | | | | | | | c 26.0 | | | | | | |
| 6 | G. S. no. 3 | 20′ 33″ | 4.24 | 17.23 | 1.17 | 578.4 | 677.6 | 465 | 213 | +286 | | | | 126 | 2,782 | +347 | 3,129 |
| 7 | G. S. no. 4 | 22′ 16″ | 3.64 | 17.63 | 1.12 | 519.4 | 579.4 | 417 | 162 | +286 | | | | | 2,498 | +347 | 2,845 |
| 8 | A. S. no. 3 | 22′ 58″ | | | | | | | | | 35.3 | 85.95 | 7.50 | | | | |
| 9 | G. S. no. 5 | 23′ 29″ | 3.36 | 17.95 | 1.14 | 468.1 | 533.8 | 376.2 | 158 | +33 | | | | 114 | 2,251 | +40 | 2,291 |
| 10 | G. S. no. 6 | 29′ 45″ | 2.83 | 18.03 | 0.96 | 463.5 | 445.9 | 372.8 | 73 | +33 | | | | 100 | 2,228 | +40 | 2,268 |
| 11 | A. S. no. 4 | 30′ 18″ | | | | | | | | | 36.9 | 75.99 | 7.52 | | | | |
| 12 | G. S. no. 7 | 35′ 53″ | 2.64 | 18.08 | 0.89 | 468 | 414 | 376.2 | 38 | −46 | | | | 100 | 2,250 | −56 | 2,194 |
| 13 | A. S. no. 5 | 36′ 28″ | | | | | | | | | 35.0 | 70.37 | 7.51 | | | | |
| 14 | G. S. no. 8 | 52′ 0″ | 2.60 | 18.17 | 0.91 | 450 | 409.1 | 361.8 | 47 | +64 | | | | 95 | 2,162 | +78 | 2,240 |
| 15 | A. S. no. 6 | 53′ 6″ | | | | | | | | | 42.1 | 62.55 | 7.46 | | | | |
| 16 | G. S. no. 9 | 66′ 20″ | 2.58 | 18.27 | 0.94 | 431.5 | 405 | 347 | 58 | +127 | | | | 90 | 2,074 | +154 | 2,228 |
| 17 | A. S. no. 7 | 67′ 16″ | | | | | | | | | 54 | 61.32 | 7.44 | | | | |
| 18 | V. S. pH | 70′ 18″ | | | | | | | | | | | | | | | |

| | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 19 | M/6 HCl 300 cc. | 71′ 20″ | | | | | | | | | c 58 | | | 86 | | | |
| 20 | G. S. no. 10 | 73′ 49″ | 3.38 | 18.11 | 1.24 | 433.9 | 537.1 | 349 | 188 | −105 | | | | 106 | 2,083 | −128 | 1,955 |
| 21 | G. S. no. 11 | 76′ 50″ | 3.65 | 18.04 | 1.33 | 438.0 | 582.9 | 352 | 231 | −105 | | | | | 2,106 | −128 | 1,978 |
| 22 | A. S. no. 8 | 77′ 28″ | | | | | | | | | 53.7 | 40.50 | 7.15 | | | | |
| 23 | G. S. no. 12 | 78′ 3″ | 3.35 | 18.04 | 1.19 | 448.9 | 532.4 | 361 | 171 | +85 | | | | 116 | 2,158 | +107 | 2,265 |
| 24 | G. S. no. 13 | 80′ 43″ | 2.98 | 18.23 | 1.11 | 423.4 | 469.9 | 340.4 | 130 | +85 | | | | | 2,037 | +107 | 2,144 |
| 25 | G. S. no. 14 | 84′ 36″ | 2.81 | 18.28 | 1.05 | 420.1 | 441.6 | 337.8 | 104 | +85 | | | | | 2,020 | +107 | 2,127 |
| 26 | A. S. no. 9 | 85′ 10″ | | | | | | | | | 58.0 | 48.29 | 7.31 | 125 | | | |
| 27 | G. S. no. 15 | 95′ 24″ | 2.71 | 18.33 | 1.03 | 413.1 | 426.0 | 332 | 94 | +85 | | | | 115 | 1,986 | +107 | 2,093 |
| 28 | A. S. no. 10 | 96′ 8″ | | | | | | | | | 64.4 | 47.50 | 7.30 | | | | |
| 29 | G. S. no. 16 | 104′ 25″ | 2.49 | 18.53 | 1.03 | 381.6 | 391.0 | 306.6 | 84 | +29 | | | | 98 | 1,835 | +35 | 1,870 |
| 30 | A. S. no. 11 | 105′ 5″ | | | | | | | | | 66.1 | 47.38 | 7.29 | | | | |

In experiment 4 there are two injections, one of sodium bicarbonate equivalent to 224.00 cc. of carbon dioxide per kgm. and the second of hydrochloric acid equivalent to 56 cc. of carbon dioxide per kgm. The non-oxidative carbon dioxide eliminated from the moment of injection of the bicarbonate to the beginning of injection of hydrochloric acid equals 74.64 cc. per kgm or 33 per cent of the carbon dioxide equivalent of the bicarbonate injected. The increase in lactic acid for the same period is equivalent to 80 cc. of carbon dioxide or 36 per cent of the carbon dioxide equivalent of the bicarbonate. The carbon dioxide index (74.64 over

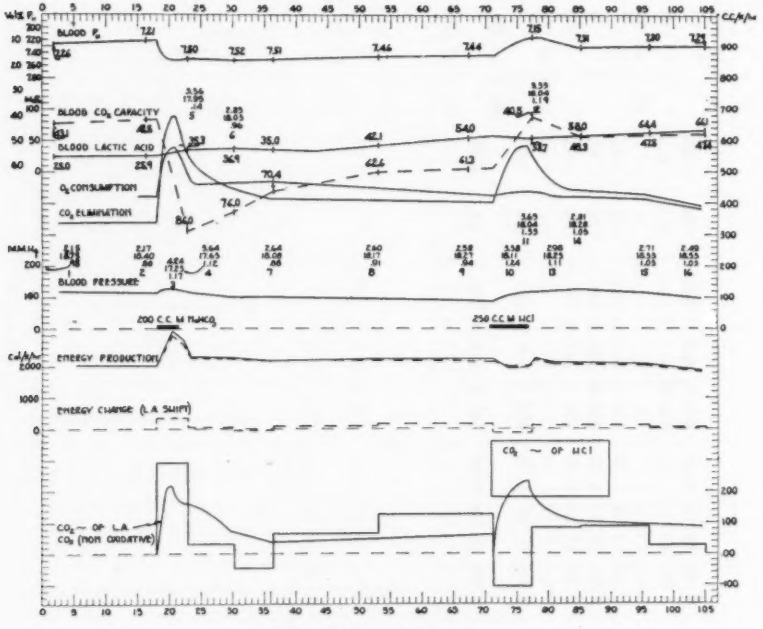



Fig. 4. Experiment 4

80.00) is 0.93 agreeing with the high figures usually obtained with bicarbonate injection. The carbon dioxide capacity of the blood before bicarbonate injection was 50.65 volumes per cent and after injection 75.75 volumes per cent giving an increase of 25.10 volumes per cent. The expected increase assuming equal distribution and no excretion is 24.40 volumes per cent.

The non-oxidative carbon dioxide from the beginning of injection of hydrochloric acid to gas sample 16 was 72.19 cc. per kgm. Since the equivalent of the hydrochloric acid was 56 cc. per kgm. the effectiveness

of the hydrochloric acid to combine with bicarbonate base is 72.19 over 56.00 129 per cent. But taking into account the accumulation of lactic acid which is equivalent to 20 cc. per kgm. the carbon dioxide index becomes 72.19 over 56.00 + 20.00 which is equal to 0.95. This is an exceptionally high figure and upholds the view that the amount of bicarbonate present determines the amount which will react with acid whether the acid be formed in the body or administered from without. The carbon dioxide capacity of the blood immediately preceding the injection of

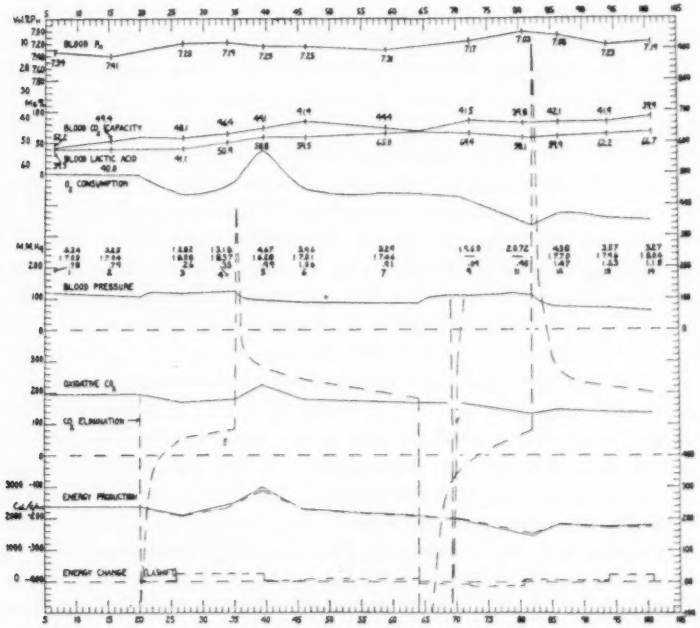



Fig. 5. Experiment 5

hydrochloric acid was 75.75 volume per cent and after injection at gas sample 16 it was 45.51 volume per cent. The decrease was 30.24 volume per cent as compared with a net decrease expected from hydrochloric and lactic acid of 5.60 + 2.00 = 7.6 volume per cent. Therefore, approximately four times as much acid stayed in the blood as was to be expected assuming even distribution of acid.

In experiment 5 the administration of carbon dioxide is substituted for the injection of hydrochloric acid. The first administration consisted of a 11.46 per cent carbon dioxide mixture in 21.40 per cent oxygen and the

second administration of a 19.22 per cent carbon dioxide mixture in 27.12 per cent oxygen. The results in many respects were similar to those produced by injection of hydrochloric acid. There was a marked reduction in oxidations with both administrations. Despite the higher oxygen pressure in the second mixture, oxidations are depressed the most. Obviously acidity is an important factor controlling oxidations. The results of administration of hydrochloric acid and carbon dioxide thus confirm findings of others on the depressant action of increased hydrogen ion concentration on oxidations in numerous forms of life. During the early period of administration elimination of carbon dioxide is replaced by a very rapid absorption which in a few minutes gives way to slower absorption as the blood and tissues approach saturation. At gas sample 3 carbon dioxide is again eliminated but at a subnormal rate. The expiratory quotient has risen to 0.38 indicating that retention of carbon dioxide formed in the body has replaced absorption from without. On readministration of room air after the first administration of carbon dioxide there is a sudden outpouring of carbon dioxide and a temporary but pronounced overshooting of oxygen absorption. After the second readministration of room air there is only a tendency towards overshooting. Oxygen consumption remains well below normal. Contrary to our experiments with hydrochloric acid and to the experiments of Anrep and Cannon with carbon dioxide, blood lactic acid failed to diminish. Instead, in this particular experiment, there is a rather rapid accumulation of lactic acid. These results agree with those of Swingle, Wenner and Stanley (1928) in which they administer carbon dioxide to the unanesthetized parathyroidectomized dog. Apparently the inhibitive action which carbon dioxide has on the accumulation of lactic acid is insufficient to cope with the augmenting effects of impaired oxidations. The increase in blood lactic acid agrees roughly with the decrease in blood carbon dioxide capacity. For the tabulated results of Experiment 5 see table 3 of the cyanide series.

To get further information on the mechanism of accumulation of lactic acid six experiments were done in which carbon dioxide was administered along with the intravenous injection of sodium bicarbonate. These administrations were so controlled as to keep the blood slightly more acid than normal. This was possible with the use of the continuous method of recording blood acidity changes with the manganese dioxide electrode. Experiment 6 (see table 5 of this series) shows effects of two administrations of carbon dioxide (11.42 per cent in a 21.08 per cent oxygen mixture) once by itself and once with simultaneous injection of sodium bicarbonate. Consulting figure 6 which gives the pH curve as established with the quinhydrone electrode it will be seen that the first administration was accompanied by a relatively large increase in hydrogen ion concentration of the blood of 0.21 pH, whereas the second administration was accom-

panied by an increased acidity of approximately 0.02 to 0.03 pH. Oxidations were reduced with both administrations suggesting that the tissues as well as the blood turned acid in both instances. During the first administration of carbon dioxide blood lactic acid showed the common tendency to diminish, whereas with the second administration it showed a distinct increase. It would thus appear that neither rate of oxidation nor blood and tissue acidity were the primary determining factors controlling the lactic acid equilibrium under these particular conditions. The results seem to support the view expressed in the preceding paper suggesting an

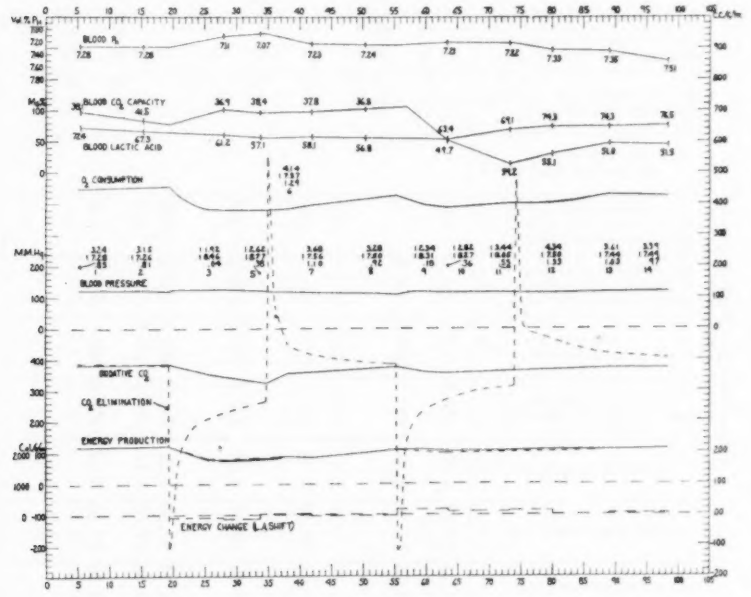



Fig. 6. Experiment 6

interchange of bicarbonate and lactate anions across the cell membrane. The absence of further increase in lactic acid with the sudden decrease in blood alkalinity from 7.22 to 7.51 on readministration of room air is also interesting in this connection. Two other experiments give the same support but in the remaining three experiments the evidence is not so strong. The increase in blood lactic acid is very small.

It is interesting to note that subnormal oxidations during the administration of hydrochloric acid and of carbon dioxide may be followed by hyperoxidations on readministration of room air just as impaired oxidations

TABLE 5

*Experiment 6. Carbon dioxide*

| OBSERVATION | PROCEDURE | TIME | PER CENT OF EXPIRED $CO_2$ | PER CENT OF EXPIRED $O_2$ | EXPIRATORY QUOTIENT | $O_2$ CONSUMPTION IN CC. PER KGM. HR. | $CO_2$ ELIMINATION IN CC. PER KGM. HR. | $CO_2$ ELIMINATION FROM OXIDATIONS BASED ON E.Q. IN CC. PER KGM. HR. | DIFFERENCE BETWEEN ACTUAL AND THEORETICAL $CO_2$ ELIMINATION | $CO_2$ EQUIVALENT OF CHANGE OF LACTIC ACID | LACTIC ACID IN MGM. PER 100 CC. ARTERIAL BLOOD | $CO_2$ CAPACITY OF ARTERIAL BLOOD IN VOLUME PER CENT | pH OF ARTERIAL BLOOD | MEAN BLOOD PRESSURE | OXIDATIVE ENERGY IN SMALL CALORIES PER KGM. HR. | NON-OXIDATIVE ENERGY IN SMALL CALORIES PER KGM. HR. | OXIDATIVE + NON-OXIDATIVE ENERGY IN SMALL CALORIES PER KGM. HR. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 |
| 1 | G. S. no. 1 | 4′ 57″ | 3.24 | 17.28 | 0.85 | 447.4 | 378.7 | 364 | +14 | | | | | 123 | 2,172 | | |
| 2 | A. S. no. 1 | 5′ 28″ | | | | | | | | | 72.4 | 38.10 | 7.28 | | | | |
| 3 | G. S. no. 2 | 14′ 53″ | 3.15 | 17.26 | 0.81 | 453 | 368.5 | 368.5 | 0 | | | | | 123 | 2,200 | | |
| 4 | A. S. no. 2 | 15′ 21″ | | | | | | | | | 67.3 | 41.51 | 7.28 | | | | |
| 5 | 11.42% $CO_2$, 21.08% $O_2$ | 19′ 20″ | | | | | | | | | c 65.2 | | | 120 | | | |
| 6 | G. S. no. 3 | 25′ 33″ | 11.92 | 18.46 | 0.04 | 383.2 | 16.4 | 309.6 | −293 | | | | | 125 | 1,862 | −83 | 1,779 |
| 7 | G. S. no. 4 (lost) | 27′ 59″ | | | | | | | | | | | | 126 | | | |
| 8 | A. S. no. 3 | 28′ 3″ | | | | | | | | | 61.2 | 36.93 | 7.11 | | | | |
| 9 | G. S. no. 5 | 33′ 42″ | 12.62 | 18.77 | 0.38 | 315.3 | 118.5 | 256.2 | −138 | | | | | 122 | 1,531 | −130 | 1,401 |
| 10 | A. S. no. 4 | 33′ 48″ | | | | | | | | | 57.1 | 38.37 | 7.07 | | | | |
| 11 | Room air | 34′ 45″ | | | | | | | | | | | | | | | |
| 12 | G. S. no. 6 | 38′ 10″ | 4.14 | 17.57 | 1.29 | 384.0 | 494.3 | 312.2 | +182 | | | | | 118 | 1,865 | +22 | 1,887 |
| 13 | G. S. no. 7 | 41′ 51″ | 3.68 | 17.56 | 1.10 | 396.7 | 435.6 | 322.3 | +113 | | | | | 117 | 1,927 | +22 | 1,949 |
| 14 | A. S. no. 5 | 41′ 56″ | | | | | | | | | 58.1 | 37.84 | 7.23 | | | | |
| 15 | G. S. no. 8 | 50′ 20″ | 3.28 | 17.50 | 0.92 | 417.6 | 386 | 339.6 | +46 | | | | | 113 | 2,030 | −28 | 2,002 |
| 16 | A. S. no. 6 | 50′ 27″ | | | | | | | | | 56.8 | 36.81 | 7.24 | | | | |
| 17 | 11.42% $CO_2$, 21.08% $O_2$ | 55′ 17″ | | | | | | | | | c 56.1 | | | 110 | | | |

| | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 18 | $NaHCO_3$ 50 cc. | 56′ 3″ | | | | | | | | | | | | | | | |
| 19 | G. S. no. 9 | 59′ 42″ | 12.34 | 18.31 | 0.18 | 396.3 | 71 | 322.2 | −251 | | | | | 118 | 1,925 | +169 | 2,094 |
| 20 | G. S. no. 10 | 63′019″ | 12.82 | 18.27 | 0.36 | 386.9 | 138 | 314.3 | +176 | | | | | 116 | 1,880 | +169 | 2,049 |
| 21 | A. S. no. 7 | 63′ 32″ | | | | | | | | | 63.4 | 49.73 | 7.21 | | | | |
| 22 | 50 cc. $NaHCO_3$ | 63′ 55″ | | | | | | | | | | | | | | | |
| 23 | G. S. no. 11 | 73′ 12″ | 13.44 | 18.05 | 0.55 | 400.6 | 221.5 | 325.7 | −104 | | | | | 116 | 1,945 | +106 | 2,051 |
| 24 | A. S. no. 8 | 73′ 20″ | | | | | | | | | 69.1 | 59.17 | 7.22 | | | | |
| 25 | Room air | 74′ 0″ | | | | | | | | | | | | | 1,944 | +141 | 2,085 |
| 26 | G. S. no. 12 | 79′ 43″ | 4.34 | 17.50 | 1.33 | 400.5 | 534.2 | 325.6 | +209 | | | | | 114 | | | |
| 27 | A. S. no. 9 | 80′ 40″ | | | | | | | | | 74.3 | 55.09 | 7.33 | | | | |
| 28 | G. S. no. 13 | 89′ 0″ | 3.61 | 17.44 | 1.03 | 428.6 | 439.7 | 348.3 | +91 | | | | | 118 | 2,081 | 0 | 2,081 |
| 29 | A. S. no. 10 | 89′ 7″ | | | | | | | | | 74.3 | 50.98 | 7.35 | | | | |
| 30 | G. S. no. 14 | 98′ 23″ | 3.39 | 17.49 | 0.97 | 424.2 | 409.5 | 345 | +64 | | | | | 120 | 2,060 | +43 | 2,103 |
| 31 | A. S. no. 11 | 98′ 28″ | | | | | | | | | 76.5 | 51.49 | 7.51 | | | | |
| 32 | Stopped drum | 99′ 20″ | | | | | | | | | | | | | | | |
| 33 | Started drum | | | | | | | | | | | | | | | | |
| 34 | G. S. no. 15 | 116′ 43″ | 3.19 | 17.53 | 0.90 | 425.1 | 383.6 | 345.7 | +38 | | | | | 118 | 2,067 | −15 | 2,052 |
| 35 | A. S. no. 12 | 116′ 41″ | | | | | | | | | 75.0 | 48.87 | 7.34 | | | | |
| 36 | G. S. no. 16 | 126′ 31″ | 3.30 | 17.37 | 0.89 | 450.2 | 402.1 | 366 | 36 | | | | | 116 | 2,188 | +110 | 2,298 |
| 37 | A. S. no. 13 | 127′ 10″ | | | | | | | | | 81.3 | 46.19 | 7.33 | | | | |
| 38 | 50 cc. $NaHCO_3$ | 127′ 43″ | | | | | | | | c | 81.6 | | | | | | |
| 39 | G. S. no. 17 | 136′ 16″ | 3.59 | 17.25 | 0.95 | 461.0 | 438.8 | 375 | 64 | | | | | 120 | 2,240 | +153 | 2,393 |
| 40 | A. S. no. 14 | 136′ 44″ | | | | | | | | | 89.6 | 57.00 | 7.40 | | | | |
| 41 | 50 cc. $NaHCO_3$ | 137′ 14″ | | | | | | | | | | | | | | | |
| 42 | G. S. no. 18 | 145′ 7″ | 3.74 | 17.20 | 0.99 | 464.7 | 457.9 | 376.8 | 81 | | | | | 123 | 2,258 | +186 | 2,444 |
| 43 | A. S. no. 15 | 145′ 27″ | | | | | | | | | 98.5 | 66.82 | 7.46 | | | | |
| 44 | G. S. no. 19 | 160′ 20″ | 3.55 | 17.25 | 0.94 | 462.6 | 433 | 376 | 57 | | | | | 104 | 2,248 | +60 | 2,308 |
| 45 | A. S. no. 16 | 160′ 58″ | | | | | | | | | 103.6 | 60.74 | 7.42 | | | | |
| 46 | G. S. no. 20 | 172′ 48″ | 3.50 | 17.28 | 0.93 | 459.4 | 426.7 | 374 | 53 | | | | | 96 | 2,231 | +66 | 2,297 |
| 47 | A. S. no. 17 | 173′ 8″ | | | | | | | | | 108.0 | 58.43 | 7.40 | | | | |

are followed by overshooting in the low oxygen, cyanide, and hemorrhage experiments. This hyperoxidation thus appears to be a compensatory reaction making up for impaired energy production of the preceding period. In the low oxygen, cyanide and hemorrhage experiments the hyperaccumulation of lactic acid consumed at an accelerated rate during recovery sufficed to explain the overshooting but in the acid experiments where lactic acid is reduced in amount (at least in the blood) this explanation does not hold. Possibly other reducing substances are formed in greater amounts. In this connection it is recalled that the production of reducing metabolites was demonstrated not only in low oxygen, cyanide and hemorrhage experiments but in experiments in carbon dioxide administration as well. On administration of carbon dioxide the manganese dioxide electrode placed in the 4th ventricle of the brain frequently gave an "alkaline" change in potential, indicating accumulation of reducing metabolites in the cerebrospinal fluid (Gesell and Hertzman, 1928).

These striking changes in oxidation reduction potential naturally suggest a regulative mechanism which conceivably contributes to the net regulation of respiration. In attempting to relate respiratory movements in this series of experiments to oxidations, however, the results so far are not very conclusive. In experiment 1 both injections produced subnormal oxidations followed by overshooting. With the first injection apnea is interrupted and respiratory movements extend slightly into the period of hyperoxidation. With the second injection apnea is again interrupted but in this instance hyperpnea extends through the period of hyperoxidation. In experiment 2 apnea was well established. There were only 4 breaths during the injection of hydrochloric acid and none following the injection of bicarbonate. The second injection which was fatal elicited hyperpnea. In experiment 3 the first injection of hydrochloric acid reduced oxidations slightly; the second injection increased them. Before the first injection of hydrochloric acid the dog was hyperpneic. This was increased and maintained for about five minutes following the end of injection. Sodium bicarbonate produced the usual momentary augmentation which then gave way to respiratory movements of smaller magnitude than those preceding bicarbonate injection agreeing with the results of the preceding paper. There was very definite stimulation during and following the second injection of hydrochloric acid. In experiment 4 bicarbonate injection momentarily excited respiratory movements which then subsided to a subpreinjection magnitude. Hydrochloric acid then stimulated respiratory movements which remained augmented. In experiment 5 the animal was markedly hyperpneic to begin with and neither administration of carbon dioxide produced an obvious effect upon the respiratory movements. In experiment 6 respiratory movements increased with both administrations, more obviously with the first, but this may be due to the fact that

hyperpnea was more marked before the second carbon dioxide administration. The experiments seem to indicate that the effects of acidity are more predominant than effects of changing oxidation yet the results are not decisive. It is proposed to study, with more precise procedure, the relation of hyperventilation to impaired oxidations as produced by limitation of oxygen and by limitation of oxidation by the administration of acid.

## SUMMARY

The effects of administration of hydrochloric acid, carbon dioxide, sodium bicarbonate and the simultaneous administration of bicarbonate and carbon dioxide were studied under experimental conditions similar to the preceding experiments.

Intravenous injection of hydrochloric acid increased the hydrogen ion concentration of the blood, decreased the blood carbon dioxide capacity and blood lactic acid. It increased the elimination of carbon dioxide and decreased the absorption of oxygen. With recovery the hydrogen ion concentration and the carbon dioxide capacity of the blood tended to approach but never reached pre-injection levels. Oxygen absorption approached and sometimes overshot preinjection values. Carbon dioxide elimination slowly approached normal levels. When oxidations were sufficiently reduced the decreasing lactic acid of the blood gave way to rapidly increasing lactic acid during acid injection.

The ratio of non-oxidative carbon dioxide to the carbon dioxide equivalent of the acid injected indicated that approximately 18 to 93 per cent of the acid injected may react with bicarbonate. Animals with previous administration of sodium bicarbonate showed the higher carbon dioxide index. It was concluded that an increase in bicarbonate content of the body leads to an increase in amount of bicarbonate reacting with the injected acid.

Computations are presented indicating that hydrochloric acid did not distribute evenly between blood and tissues; that the blood contained from 4 to 6 times as much as demanded by even distribution. This differs from the more uniform distribution of sodium bicarbonate.

The administration of carbon dioxide in 21+ per cent $O_2$ mixtures decreased oxidations and commonly decreased blood lactic acid.

Since blood lactic acid may decrease on acid injection though oxidations decrease and since blood lactic acid always increases on intravenous injection of sodium bicarbonate though oxidations are markedly increased, it is obvious that there are several factors determining lactic acid equilibrium.

When bicarbonate and carbon dioxide were simultaneously administered in proportions to keep the blood about 0.02 pH more acid than normal,

blood lactic acid increased decidedly in three experiments. In three other experiments the increase was very small.

It is suggested that the increase in blood lactic acid under these conditions may be due in part to an increase in the $HCO_3$ ions which cross the cell membrane to exchange places with the lactate ions which in turn enter the blood.

Since stimulation of respiratory movements by intravenous injection of sodium bicarbonate may sooner or later give way to a sub-preinjection magnitude it is suggested that sodium bicarbonate also crosses the membrane. This agrees with our findings that sodium bicarbonate distributes itself with relative uniformity between the blood and tissues.

The relation of respiratory movements to oxidations and acidity is further discussed.

## BIBLIOGRAPHY

ANREP AND CANNON. 1923–24. Journ. Physiol., lviii, 244.
HALDANE. 1921. Journ. Physiol., lv, 265.
GESELL AND HERTZMAN. 1928. This Journal, lxxxvii, 24.
SWINGLE, WENNER AND STANLEY. 1928. Physiol. Zoöl., i, 172.

# THE REGULATION OF RESPIRATION

## A Study of the Correlation of Numerous Factors in Respiratory Control Produced by Intravenous Injection of Ammonium Chloride, Methylene Blue, Strychnine and Sodium Sulphide[1]

ROBERT GESELL, HUGO KRUEGER,[2] GRACE GORHAM AND THEODORE BERNTHAL

*From the Department of Physiology, University of Michigan, Ann Arbor*

Received for publication March 17, 1930

The ingestion of ammonium chloride in man is rapidly followed by a reduction in the carbon dioxide content of the blood, an increase in the hydrogen ion concentration of the blood, a decrease in alveolar carbon dioxide pressure, an increase in pulmonary ventilation and a feeling of air hunger on exertion (Haldane, 1921; Koehler, 1927). It seemed desirable to compare ammonium chloride acidosis with the acid base disturbances described in the preceding papers. Accordingly the effects of intravenous injection of ammonium chloride were studied under experimental conditions employed throughout this series.

Results of three of these experiments are shown in figures 1, 2 and 3 and tables 1, 2 and 3. It is apparent that we have obtained a similar increase in the hydrogen ion concentration of the blood and a similar decrease in the blood carbon dioxide capacity as occurs in man. The changes in alveolar carbon dioxide pressure are somewhat different but two important differences in experimental procedure might easily account for this. In the first place ammonium chloride was undoubtedly administered more rapidly than the rate of absorption from the gastro-intestinal tract in man, and secondly, pulmonary ventilation was held at a constant volume. In the first two experiments, in which ammonium chloride was injected more rapidly than in the third experiment, there was first a momentary augmentation of oxygen absorption and carbon dioxide elimination, then a temporary depression to subnormal levels followed again by a supernormal gaseous exchange which eventually returned toward normal. The

[1] This investigation has been made with the assistance of a grant from the Committee on Therapeutic Research, Council on Pharmacy and Chemistry, American Medical Association.

[2] Submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

TABLE 1

*Experiment 1. Ammonium chloride and methylene blue*

| OBSERVATION | PROCEDURE | TIME | PER CENT OF EXPIRED $CO_2$ | PER CENT OF EXPIRED $O_2$ | EXPIRATORY QUOTIENT | $O_2$ CONSUMPTION IN CC. PER KGM. HR. | $CO_2$ ELIMINATION IN CC. PER KGM. HR. | $CO_2$ ELIMINATION FROM OXIDATIONS BASED ON E. Q. IN CC. PER KGM. HR. | DIFFERENCE BETWEEN ACTUAL AND THEORETICAL $CO_2$ ELIMINATION | $CO_2$ EQUIVALENT OF CHANGE OF LACTIC ACID | LACTIC ACID IN MGM. PER 100 CC. ARTERIAL BLOOD | $CO_2$ CAPACITY OF ARTERIAL BLOOD IN VOL. PER CENT | pH OF ARTERIAL BLOOD | MEAN BLOOD PRESSURE | OXIDATIVE ENERGY IN SMALL CALORIES PER KGM. HR. | NON-OXIDATIVE ENERGY IN SMALL CALORIES PER KGM. HR. | OXIDATIVE + NON-OXIDATIVE ENERGY IN SMALL CALORIES PER KGM. HR. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 |
| 1 | G. S. no. 1 | 14′ 25″ | 2.82 | 17.90 | 0.89 | 357.3 | 319.4 | 313.4 | +6 | | | | | 105 | 1,750 | | |
| 2 | A. S. no. 1 | 15′ 13″ | | | | | | | | | 43.8 | 47.76 | 7.36 | | | | |
| 3 | G. S. no. 2 | 26′ 0″ | 2.68 | 18.01 | 0.88 | 342.7 | 300.5 | 300.5 | | | | | | 97 | 1,677 | | |
| 4 | A. S. no. 2 | 26′ 47″ | | | | | | | | | 43.7 | 47.22 | 7.36 | | | | |
| 5 | n/$NH_4Cl$ 42 cc. | 29′ 12″ | | | | | | | | | c 43.7 | | | 93 | | | |
| 6 | G. S. no. 3 | 32′ 55″ | 2.81 | 17.83 | 0.87 | 364.1 | 315.1 | 319.2 | −4 | −20 | | | | 98 | 1,782 | −25 | 1,757 |
| 7 | G. S. no. 4 | 36′ 26″ | 2.68 | 18.11 | 0.92 | 328.3 | 300.9 | 288 | +13 | −20 | | | | 72 | 1,607 | −25 | 1,582 |
| 8 | G. S. no. 5 | 37′ 45″ | 2.58 | 18.39 | 0.99 | 291.3 | 289.5 | 255.3 | +34 | −20 | | | | 70 | 1,426 | −25 | 1,401 |
| 9 | A. S. no. 3 | 38′ 4″ | | | | | | | | | 42.5 | 37.11 | 7.22 | | | | |
| 10 | G. S. no. 6 | 45′ 41″ | 2.40 | 18.47 | 0.94 | 285.2 | 269.3 | 250.1 | +19 | +52 | | | | 58 | 1,396 | +63 | 1,459 |
| 11 | A. S. no. 4 | 46′ 27″ | | | | | | | | | 45.4 | 36.51 | 7.22 | | | | |
| 12 | G. S. no. 7 | 49′ 45″ | 2.83 | 18.06 | 0.96 | 331.0 | 318.4 | 290 | +28 | +367 | | | | 80 | 1,620 | +446 | 2,066 |
| 13 | G. S. no. 8 | 52′ 47″ | 3.23 | 17.70 | 0.96 | 366.0 | 351.1 | 321 | +30 | +367 | | | | 97 | 1,791 | +446 | 2,237 |
| 14 | A. S. no. 5 | 52′ 57″ | | | | | | | | | 60.8 | 32.11 | 7.15 | | | | |
| 15 | G. S. no. 9 | 55′ 30″ | 3.31 | 17.76 | 1.04 | 356.5 | 368.9 | 312.5 | +57 | −23 | | | | 100 | 1,744 | −28 | 1,716 |
| 16 | G. S. no. 10 | 59′ 10″ | 3.09 | 18.09 | 1.09 | 316.3 | 344.6 | 277.3 | +67 | −23 | | | | 96 | 1,548 | −28 | 1,520 |
| 17 | A. S. no. 6 | 59′ 21″ | | | | | | | | | 59.8 | 36.10 | 7.15 | | | | |
| 18 | G. S. no. 11 | 68′ 54″ | 2.82 | 18.29 | 1.06 | 298.8 | 317.0 | 262 | +53 | −22 | | | | 85 | 1,461 | −26 | 1,435 |
| 19 | A. S. no. 7 | 69′ 25″ | | | | | | | | | 58.3 | 34.08 | 7.16 | | | | |

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 20 | G. S. no. 12 | 80′ 1″ | 2.93 | 18.37 | 1.16 | 284.7 | 330.4 | 249.5 | 81 | +64 | | | | 94 | 1,394 | +77 | 1,471 |
| 21 | A. S. no. 8 | 80′ 1″ | | | | | | | | | 62.8 | 34.29 | 7.17 | | | | |
| 22 | 0.025 n meth. blue, 100 cc. | 83′ 30″ | | | | | | | | | c 64.4 | | | | | | |
| 23 | G. S. no. 13 | 87′ 42″ | 3.25 | 17.96 | 1.10 | 340.5 | 374.3 | 298.5 | 76 | +98 | | | | 104 | 1,666 | +120 | +1,786 |
| 24 | G. S. no. 14 | 89′ 56″ | 3.58 | 17.77 | 1.15 | 358.7 | 413.6 | 314.4 | 99 | +98 | | | | 110 | 1,755 | +120 | 1,875 |
| 25 | A. S. no. 9 | 90′ 13″ | | | | | | | | | 68.8 | 33.71 | 7.10 | | | | |
| 26 | G. S. no. 15 | 91′ 53″ | 3.44 | 17.94 | 1.18 | 337.8 | 397.4 | 296 | 101 | −159 | | | | 98 | 1,653 | −193 | 1,460 |
| 27 | G. S. no. 16 | 95′ 58″ | 3.04 | 18.29 | 1.17 | 299.0 | 350.4 | 262.2 | 88 | −159 | | | | 85 | 1,464 | −193 | 1,271 |
| 28 | G. S. no. 17 | 100′ 18″ | 2.74 | 18.40 | 1.08 | 290.1 | 314.6 | 254.2 | 60 | −159 | | | | 86 | 1,420 | −193 | 1,227 |
| 29 | A. S. no. 10 | 100′ 15″ | | | | | | | | | 58.2 | 34.40 | 7.14 | | | | |

significance of these changes which invariably followed the more rapid injections is not entirely clear. It will be seen that they parallel rather closely the changes in mean blood pressure which are in turn associated with similar changes in the volume flow of blood through the carotid artery. The magnitude of the respiratory movements as a rule varied roughly with the magnitude of the gaseous exchange. The marked depression of respiratory movements during the period of impaired oxidation stands in contrast to augmented ventilation during impaired oxidations of hemorrhage, lowered alveolar oxygen and cyanide administration. In experiment

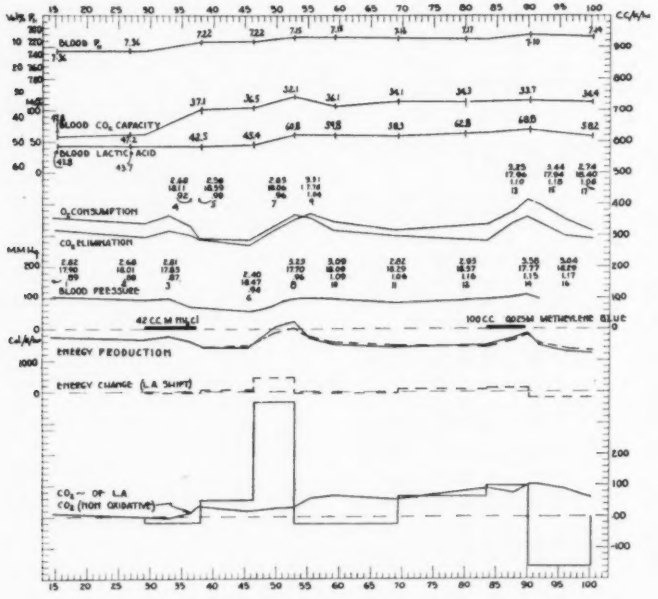



Fig. 1. Experiment 1

3 in which ammonium chloride was administered more slowly the initial augmentation of gaseous exchange was missing and the secondary augmentation was relatively small.

The immediate effects of ammonium chloride on blood lactic acid are uncertain. In some experiments there was a minor decrease and in others an increase. In this respect the results differ from the more constant effects of administration of carbon dioxide and hydrochloric acid of the preceding experiments. A late increase in blood lactic acid beginning with the increase in oxidations was more common. This occurred

in six out of seven experiments. In experiment 3 in which ammonium chloride was injected more slowly than in the other experiments there was a net reduction in blood lactic acid. The amount of lactic acid formed per unit of oxygen shortage per kilogram of body weight was as a rule relatively small as indicated by the curve of energy production. Decreased oxidations were always associated with a decrease in total energy production.

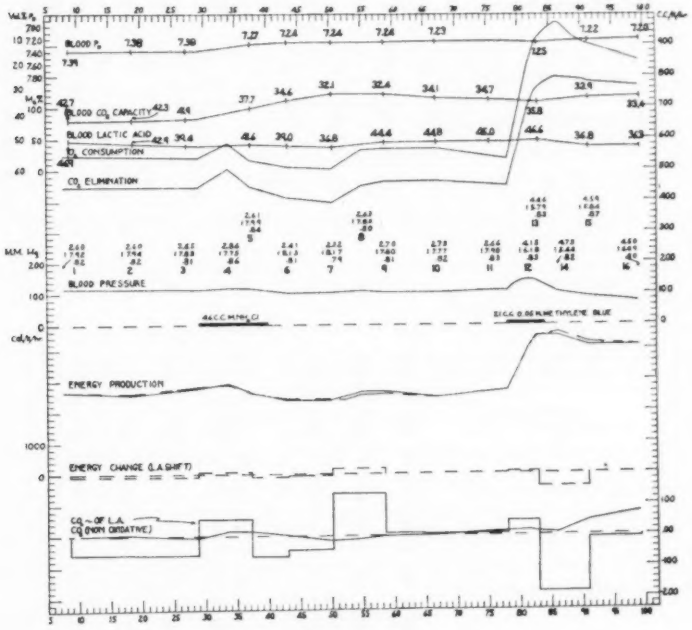



Fig. 2. Experiment 2

According to Haldane the acidosis produced by the ingestion of ammonium chloride is associated with an elimination of the ammonia radicle in the form of urea and a corresponding liberation of free hydrochloric acid which then reacts with the buffers of the body. From his experiments he concluded that the hydrochloric acid so derived was about twice as concentrated in the blood as in the soft tissues. Our experiments show a similar uneven distribution of hydrochloric acid. In experiment 1 the carbon dioxide equivalent of the ammonium chloride injected was 43.40 cc. per kilogram of body weight. The carbon dioxide capacity of the blood immediately preceding injection was 47.00 volumes per cent and the carbon

TABLE 2

*Experiment 2. Ammonium chloride and methylene blue*

| OBSERVATION | PROCEDURE | TIME | PER CENT OF EXPIRED $CO_2$ | PER CENT OF EXPIRED $O_2$ | EXPIRATORY QUOTIENT | $O_2$ CONSUMPTION IN CC. PER KGM. HR. | $CO_2$ ELIMINATION IN CC. PER KGM. HR. | $CO_2$ ELIMINATION FROM OXIDATIONS BASED ON E.Q. IN CC. PER KGM. HR. | DIFFERENCE BETWEEN ACTUAL AND THEORETICAL $CO_2$ ELIMINATION | $CO_2$ EQUIVALENT OF CHANGE OF LACTIC ACID | LACTIC ACID IN MGM. PER 100 CC. ARTERIAL BLOOD | $CO_2$ CAPACITY OF ARTERIAL BLOOD IN VOL. PER CENT | pH OF ARTERIAL BLOOD | MEAN BLOOD PRESSURE | OXIDATIVE ENERGY IN SMALL CALORIES PER KGM. HR. | NON-OXIDATIVE ENERGY IN SMALL CALORIES PER KGM. HR. | OXIDATIVE + NON-OXIDATIVE ENERGY IN SMALL CALORIES PER KGM. HR. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 |
| 1 | G. S. no. 1 | 7′ 33″ | 2.60 | 17.92 | 0.82 | 547.0 | 446.0 | 446 | | | | | | 118 | 2,636 | | |
| 2 | A. S. no. 1 | 8′ 35″ | | | | | | | | | 46.9 | 42.71 | 7.39 | | | | |
| 3 | G. S. no. 2 | 18′ 15″ | 2.60 | 17.94 | 0.82 | 543.7 | 446.1 | 443 | +3 | −60 | | | | 117 | 2,618 | −73 | 2,545 |
| 4 | A. S. no. 2 | 18′ 36″ | | | | | | | | | 42.9 | 42.28 | 7.38 | | | | |
| 5 | G. S. no. 3 | 26′ 35″ | 2.65 | 17.83 | 0.81 | 578.8 | 466.2 | 471 | −5 | −61 | | | | 116 | 2,789 | −75 | 2,714 |
| 6 | A. S. no. 3 | 27′ 6″ | | | | | | | | | 39.4 | 41.94 | 7.38 | | | | |
| 7 | n/$NH_4Cl$, 46 cc. | 28′ 55″ | | | | | | | | | c 38.4 | | | 115 | | | |
| 8 | G. S. no. 4 | 33′ 42″ | 2.86 | 17.75 | 0.86 | 588.6 | 504.5 | 488 | +16 | +57 | | | | 120 | 2,837 | +69 | 2,906 |
| 9 | G. S. no. 5 | 37′ 15″ | 2.61 | 17.99 | 0.84 | 532.1 | 448.4 | 433.6 | +15 | +57 | | | | 118 | 2,564 | +69 | 2,633 |
| 10 | A. S. no. 4 | 37′ 21″ | | | | | | | | | 41.6 | 37.67 | 7.27 | | | | |
| 11 | G. S. no. 6 | 43′ 5″ | 2.41 | 18.13 | 0.81 | 510.8 | 413.2 | 416.2 | −2 | −67 | | | | 103 | 2,461 | −82 | 2,379 |
| 12 | A. S. no. 5 | 43′ 8″ | | | | | | | | | 39.0 | 34.58 | 7.24 | | | | |
| 13 | G. S. no. 7 | 50′ 5″ | 2.32 | 18.17 | 0.79 | 504.0 | 396.2 | 411 | −15 | −46 | | | | 105 | 2,428 | −56 | 2,372 |
| 14 | A. S. no. 6 | 50′ 15″ | | | | | | | | | 36.8 | 32.10 | 7.24 | | | | |
| 15 | G. S. no. 8 | 54′ 55″ | 2.63 | 17.83 | 0.80 | 564.6 | 449.9 | 460.5 | −11 | +137 | | | | 110 | 2,722 | +167 | 2,555 |
| 16 | G. S. no. 9 | 58′ 36″ | 2.70 | 17.80 | 0.81 | 568.3 | 462.3 | 463.2 | −1 | +137 | | | | 105 periodic | 2,740 | +167 | 2,573 |
| 17 | A. S. no. 7 | 58′ 33″ | | | | | | | | | 44.4 | 32.36 | 7.24 | | | | |

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 18 | G. S. no. 10 | 66′ 53″ | 2.73 | 17.77 | 0.82 | 569.3 | 464.1 | 404 | 0 | +7 | | | | 105 periodic | 2,742 | +9 | 2,751 |
| 19 | A. S. no. 8 | 66′ 53″ | | | | | | | | | 44.8 | 34.10 | 7.23 | | | | |
| 20 | G. S. no. 11 | 75′ 21″ | 2.66 | 17.90 | 0.83 | 543.9 | 452.6 | 443 | −10 | +4 | | | | 110 periodic | 2,620 | +4 | 2,624 |
| 21 | A. S. no. 9 | 75′ 21″ | | | | | | | | | 45.0 | 34.73 | | | | | |
| 22 | 0.05 n meth. blue, 21 cc. | 78′ 5″ | | | | | | | | | c 45.1 | | | 110 | | | |
| 23 | G. S. no. 12 | 81′ 28″ | 4.15 | 16.18 | 0.83 | 840.3 | 700.6 | .685 | +16 | +46 | | | | 145 | 4,050 | +56 | 4,106 |
| 24 | G. S. no. 13 | 82′ 37″ | 4.46 | 15.79 | 0.83 | 909.3 | 752.6 | 741 | +12 | +46 | | | | 138 | 4,380 | +56 | 4,436 |
| 25 | A. S. no. 10 | 83′ 0″ | | | | | | | | | 46.6 | 35.77 | 7.25 | | | | |
| 26 | G. S. no. 14 | 86′ 8″ | 4.73 | 15.44 | 0.82 | 972.2 | 797.8 | 792 | +6 | −183 | | | | 105 periodic | 4,685 | −233 | 4,452 |
| 27 | G. S. no. 15 | 91′ 7″ | 4.59 | 15.84 | 0.87 | 904.9 | 784.2 | 737 | +47 | −183 | | | | 90 periodic | 4,360 | −233 | 4,127 |
| 28 | A. S. no. 11 | 91′ 1″ | | | | | | | | | 36.8 | 33.91 | 7.22 | | | | |
| 29 | G. S. no. 16 | 99′ 8″ | 4.50 | 16.09 | 0.90 | 854.9 | 770.2 | 696.5 | +74 | −9 | | | | 76 | 4,120 | −11 | 4,109 |
| 30 | A. S. no. 12 | 99′ 20″ | | | | | | | | | 36.3 | 33.42 | 7.20 | | | | |
| | Stopped | 100′ 20″ | | | | | | | | | | | | | | | |

TABLE 3

*Experiment 3. Ammonium chloride*

| OBSERVATION | PROCEDURE | TIME | PER CENT OF EXPIRED $CO_2$ | PER CENT OF EXPIRED $O_2$ | EXPIRATORY QUOTIENT | $O_2$ CONSUMPTION IN CC. PER KGM. HR. | $CO_2$ ELIMINATION IN CC. PER KGM. HR. | $CO_2$ ELIMINATION FROM OXIDATIONS BASED ON E.Q. IN CC. PER KGM. HR. | DIFFERENCE BETWEEN ACTUAL AND THEORETICAL $CO_2$ ELIMINATION | $CO_2$ EQUIVALENT OF CHANGE OF LACTIC ACID | LACTIC ACID IN MGM. PER 100 CC. ARTERIAL BLOOD | $CO_2$ CAPACITY OF ARTERIAL BLOOD IN VOL. PER CENT | pH OF ARTERIAL BLOOD | MEAN BLOOD PRESSURE | OXIDATIVE ENERGY IN SMALL CALORIES PER KGM. HR. | NON-OXIDATIVE ENERGY IN SMALL CALORIES PER KGM. HR. | OXIDATIVE + NON-OXIDATIVE ENERGY IN SMALL CALORIES PER KGM. HR. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 |
| 1 | G. S. no. 1 | 3′ 8″ | 3.24 | 16.90 | 0.75 | 398.3 | 299.1 | 299 | | | | | | 148 | 1,888 | | |
| 2 | A. S. no. 1 | 3′ 26″ | | | | | | | | | 31.7 | 46.97 | 7.39 | | | | |
| 3 | G. S. no. 2 | 13′ 18″ | 3.25 | 16.88 | 0.75 | 400 | 300.1 | 300 | | −60 | | | | 142 | 1,896 | | |
| 4 | A. S. no. 2 | 13′ 28″ | | | | | | | | | 27.7 | 45.00 | 7.37 | | | | |
| 5 | m/$NH_4Cl$ 31 cc. | 17′ 3″ | | | | | | | | | c 26.1 | | | 140 | | | |
| 6 | G. S. no. 3 | 20′ 36″ | 3.30 | 16.93 | 0.77 | 395.6 | 306.3 | 297 | +9 | −75 | | | | 145 | 1,876 | −91 | 1,785 |
| 7 | G. S. no. 4 | 23′ 36″ | 3.23 | 17.04 | 0.78 | 384.5 | 299.9 | 288.8 | +11 | −75 | | | | 140 | 1,823 | −91 | 1,732 |
| 8 | A. S. no. 3 | 23′ 53″ | | | | | | | | | 22.7 | 40.22 | 7.31 | | | | |
| 9 | G. S. no. 5 | 32′ 55″ | 3.07 | 17.01 | 0.73 | 391.1 | 284.1 | 293.3 | −9 | +49 | | | | 135 | 1,855 | +59 | 1,914 |
| 10 | A. S. no. 4 | 32′ 50″ | | | | | | | | | 25.6 | 38.80 | 7.31 | | | | |
| 11 | G. S. no. 6 | 41′ 33″ | 3.19 | 16.96 | 0.75 | 400 | 300 | 300 | | −26 | | | | 140 | 1,896 | −31 | 1,865 |
| 12 | A. S. no. 5 | 41′ 40″ | | | | | | | | | 24.1 | 36.52 | 7.29 | | | | |
| 13 | G. S. no. 7 | 52′ 30″ | 3.12 | 17.09 | 0.76 | 386.3 | 293.5 | 290 | +4 | −15 | | | | 138 | 1,833 | −19 | 1,814 |
| 14 | A. S. no. 6 | 52′ 27″ | | | | | | | | | 23.0 | 38.75 | 7.32 | | | | |
| 15 | Nicotine 2 cc. 0.0170 | 58′ 55″ | | | | | | | | | c 22.4 | | | 137 | | | |
| 16 | G. S. no. 8 | 61′ 17″ | 3.35 | 16.63 | 0.74 | 417.3 | 308.9 | 314 | −5 | +252 | | | | 146 | 1,980 | +314 | 2,294 |
| 17 | G. S. no. 9 | 63′ 45″ | 3.26 | 16.94 | 0.77 | 394.0 | 301.3 | 296 | +5 | +252 | | | | 130 | 1,868 | +314 | 2,182 |
| 18 | A. S. no. 7 | 63′ 58″ | | | | | | | | | 30.9 | 35.82 | 7.24 | | | | |
| 19 | G. S. no. 10 | 72′ 58″ | 3.12 | 17.05 | 0.75 | 384 | 288.1 | 288 | | −93 | | | | 133 | 1,820 | −114 | 1,706 |

| | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 20 | A. S. no. 8 | 73′ 45″ | | | | | | | | | 24.8 | 37.17 | 7.27 | | | | |
| 21 | G. S. no. 11 | 86′ 11″ | 3.13 | 17.07 | 0.76 | 381 | 288.6 | 286 | +3 | +59 | | | | 130 | 1,804 | +72 | 1,876 |
| 22 | A. S. no. 9 | 86′ 9″ | | | | | | | | | 29.7 | 35.35 | 7.26 | | | | |
| 23 | 0.1 n meth. blue, 15 cc. | 89′ 40″ | | | | | | | | | c 31.0 | | | 128 | | | |
| 24 | Blank | | | | | | | | | | | | | 144 | | | |
| 25 | G. S. no. 12 | 93′ 21″ | 3.78 | 16.49 | 0.81 | 433.7 | 349.6 | 325.8 | +24 | +208 | | | | 100 | 2,058 | +253 | 2,311 |
| 26 | G. S. no. 13 | 94′ 28″ | 4.07 | 16.09 | 0.80 | 473.3 | 376.2 | 355.2 | +21 | +208 | | | | | 2,244 | +253 | 2,497 |
| 27 | A. S. no. 10 | 94′ 38″ | | | | | | | | | 37.9 | 34.22 | 7.17 | 90 | | | |
| 28 | G. S. no. 14 | 97′ 8″ | 4.35 | 15.70 | 0.79 | 511.8 | 401.8 | 384 | +18 | −49 | | | | 104 | 2,426 | −60 | 2,366 |
| 29 | G. S. no. 15 | 104′ 45″ | 4.69 | 15.70 | 0.86 | 505.5 | 435.3 | 379.5 | +56 | −49 | | | | | 2,398 | −60 | 2,338 |
| 30 | A. S. no. 11 | 104′ 52″ | | | | | | | | | 35.4 | 34.57 | 7.18 | | | | |
| 31 | Stopped drum | 107′ | | | | | | | | | | | | | | | |

dioxide capacity at gas sample 12 was 34.29 volumes per cent which gives an observed decrease of 12.71 volumes per cent. Assuming that all of the hydrochloric acid bound with ammonia is liberated and that all of the lactic acid which is formed in the body reacts with bicarbonate the net expected increase is 4.34 + 4.80 or 9.14 volumes per cent leaving 3.57 volumes per cent unaccounted for. Accepting Salkowski's finding (1878) that two-thirds of the ammonium chloride injected is eliminated as such, the difference between the observed (12.71 volumes per cent) and the expected (6.23 volumes per cent) is greater—namely, 6.48 volumes per

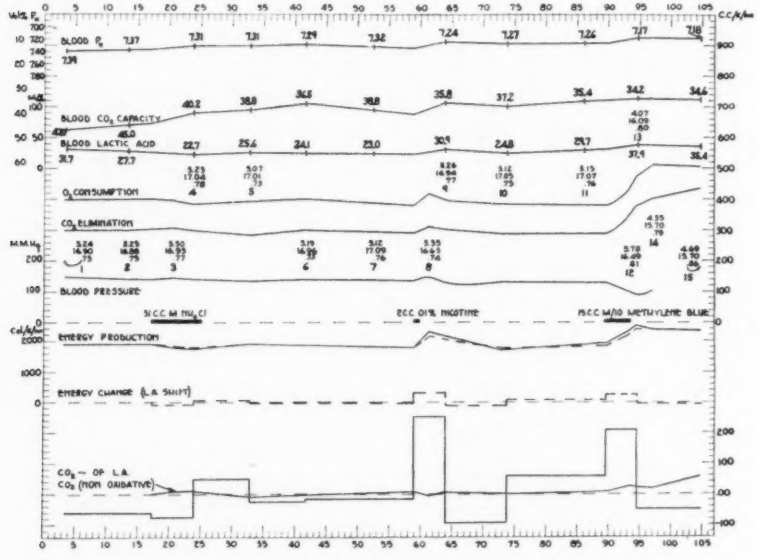



Fig. 3. Experiment 3

cent. The hydrochloric acid derived from the ammonium chloride is accordingly about twice as concentrated in the blood as in the tissues. These results agree with our earlier findings on the uneven distribution of intravenously administered hydrochloric acid between the blood and tissues. In experiment 2 the carbon dioxide capacity of the blood before injection of ammonium chloride was 41.75 volumes per cent and at gas sample 11 it was 34.73 volumes per cent giving an observed decrease of 7.02 volumes per cent. The decrease expected from complete liberation of hydrochloric acid and complete reaction with bicarbonate was 5.04 volumes per cent and from complete reaction of lactic acid with bicarbonate was 1.65 volumes per cent or a net decrease of 6.69 volumes per

cent. Again assuming a liberation of only one-third of the bound hydrochloric acid the expected decrease was only 1.66 + 1.65 or 3.31 volumes per cent.

If we are to assume that the changes in tissue acidity run parallel with the changes in blood acidity following injection of ammonium chloride there is a lack of correspondence between tissue acidity and pulmonary ventilation for it will be recalled that decreased respiratory movements prevail shortly after the initial increase and during the height of acidity of the blood. Possibly the ammonia radicle is entering the tissues leaving hydrochloric acid behind which theoretically might account for the tem-

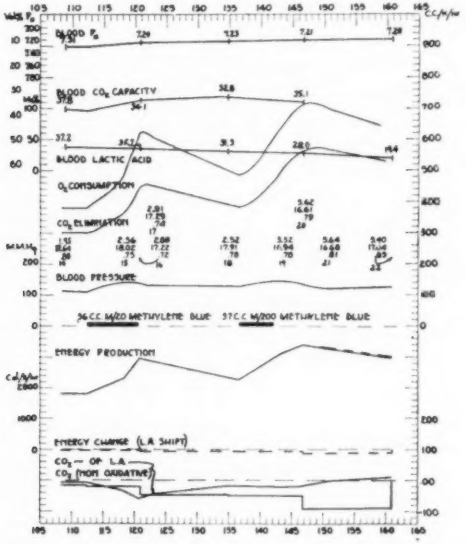



Fig. 4. Experiment 4

porary depression of respiratory movements by a decrease in acidity of the respiratory center. The peculiar irregular type of respiratory movements, however, suggests that the ammonia radicle exerts a specific action. It is thus possible that this specific action also prevents the inverse relation between rate of oxidations and pulmonary ventilation as obtains with lowered alveolar oxygen.

METHYLENE BLUE. From the work of Heymans and others it is now generally known that methylene blue is a powerful excitor of cellular oxidations and that intravenous injection produces a profound increase in oxygen consumption, carbon dioxide elimination, body temperature and

TABLE 4

*Experiment 4. Ammonium chloride*

| OBSERVATION | PROCEDURE | TIME | PER CENT OF EXPIRED $CO_2$ | PER CENT OF EXPIRED $O_2$ | EXPIRATORY QUOTIENT | $O_2$ CONSUMPTION IN CC. PER KGM. HR. | $CO_2$ ELIMINATION IN CC. PER KGM. HR. | $CO_2$ ELIMINATION FROM OXIDATIONS BASED ON E.Q. IN CC. PER KGM. HR. | DIFFERENCE BETWEEN ACTUAL AND THEORETICAL $CO_2$ ELIMINATION | $CO_2$ EQUIVALENT OF CHANGE OF LACTIC ACID | LACTIC ACID IN MGM. PER 100 CC. ARTERIAL BLOOD | $CO_2$ CAPACITY OF ARTERIAL BLOOD IN VOL. PER CENT | pH OF ARTERIAL BLOOD | MEAN BLOOD PRESSURE | OXIDATIVE ENERGY IN SMALL CALORIES PER KGM. HR. | NON-OXIDATIVE ENERGY IN SMALL CALORIES PER KGM. HR. | OXIDATIVE + NON-OXIDATIVE ENERGY IN SMALL CALORIES PER KGM. HR. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 |
| 25 | G. S. no. 14 | 108′ 15″ | 1.95 | 18.64 | 0.80 | 379.2 | 301.8 | 309 | −7 | −17 | | | | 114 | 1,828 | −20 | 1,808 |
| 26 | A. S. no. 8 | 108′ 52″ | | | | | | | | | 37.2 | 37.77 | 7.31 | | | | |
| 27 | 0.05 meth. blue, 36 cc. | 112′ 20″ | | | | | | | | | c 36.8 | | | 110 | | | |
| 28 | G. S. no. 15 | 118′ 15″ | 2.36 | 18.02 | 0.75 | 485.3 | 365 | 395.5 | −31 | −19 | | | | 140 | 2.340 | −23 | 2,317 |
| 29 | G. S. no. 16 | 120′ 42″ | 2.88 | 17.22 | 0.72 | 619.2 | 445.2 | 504 | −59 | −19 | | | | 136 | 2,985 | −23 | 2,962 |
| 30 | A. S. no. 9 | 120′ 55″ | | | | | | | | | 35.7 | 34.10 | 7.24 | | | | |
| 31 | G. S. no. 17 | 122′ 55″ | 2.91 | 17.29 | 0.74 | 605.9 | 450.5 | 494 | −44 | −47 | | | | 130 | 2,920 | −57 | 2,863 |
| 32 | G. S. no. 18 | 134′ 47″ | 2.52 | 17.91 | 0.78 | 500.6 | 390.4 | 408 | −18 | −47 | | | | 128 | 2,413 | −57 | 2,356 |
| 33 | A. S. no. 10 | 134′ 53″ | | | | | | | | | 31.3 | 32.80 | 7.23 | | | | |
| 34 | 0.05 n meth. blue, 60 cc. | 136′ 38″ | | | | | | | | | c 30.7 | | | 126 | | | |
| 35 | G. S. no. 19 | 143′ 25″ | 3.32 | 16.94 | 0.78 | 659. | 516.1 | 537.4 | −21 | −49 | | | | 143 | 3,178 | −59 | 3,119 |
| 36 | G. S. no. 20 | 146′ 39″ | 3.62 | 16.61 | 0.79 | 712.4 | 563.0 | 580.7 | −18 | −49 | | | | 135 | 3,432 | −59 | 3,373 |
| 37 | A. S. no. 11 | 146′ 49″ | | | | | | | | | 28.0 | 35.10 | 7.21 | | | | |
| 38 | G. S. no. 21 | 150′ 42″ | 3.64 | 16.68 | 0.81 | 698.3 | 566.8 | 569.3 | −3 | −91 | | | | 120 | 3,368 | −111 | 3,257 |
| 39 | G. S. no. 22 | 160′ 54″ | 3.40 | 17.04 | 0.83 | 637.2 | 529.9 | 519.2 | +10 | −91 | | | | 125 | 3,072 | −111 | 2,961 |
| 40 | A. S. no. 12 | 160′ 58″ | | | | | | | | | 19.4 | No sample | 7.20 | | | | |
| 41 | Quinhydrone injection, 550 cc. | 163′ 42″ | | | | | | | | | | | | | | | |
| 42 | Animal died | 176′ 30″ | | | | | | | | | | | | | | | |

pulmonary ventilation. The effects of severe injections studied by the methods of the preceding papers are shown in the graphs and tables of experiments 1, 2, 3, 4 and 5.

Changes common to most of the sublethal injections were an increase in the hydrogen ion concentration of the blood usually followed by a return towards normal, an increase in oxygen absorption and carbon dioxide elimination followed by recovery, a temporary increase in mean blood pressure associated with a parallel augmentation of volume flow of blood and respiratory movements. The effects of intravenous injection of

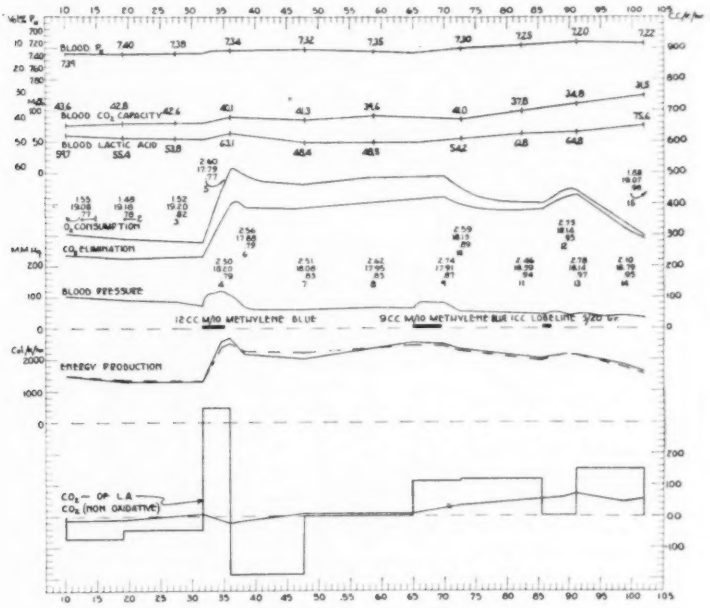



Fig. 5. Experiment 5

methylene blue on blood lactic acid were somewhat variable. A late decrease always occurred but this was frequently preceded by an initial increase of varying proportions. Whether this initial increase is to be looked upon as a temporary change in metabolism or as an increased movement of lactic acid from the tissues permitted by a change in membrane permeability is difficult to say. The late decrease in blood lactic acid possibly represents a metabolic removal of lactic acid in the cells. If this removal were brought about solely by increased oxidations an approximation of the expiratory quotient towards 1 might be expected.

TABLE 5

*Experiment 5. Methylene blue*

| OBSERVATION | PROCEDURE | TIME | PER CENT OF EXPIRED $CO_2$ | PER CENT OF EXPIRED $O_2$ | EXPIRATORY QUOTIENT | $O_2$ CONSUMPTION IN CC. PER KGM. HR. | $CO_2$ ELIMINATION IN CC. PER KGM. HR. | $CO_2$ ELIMINATION FROM OXIDATIONS BASED ON E.Q. IN CC. PER KGM. HR. | DIFFERENCE BETWEEN ACTUAL AND THEORETICAL $CO_2$ ELIMINATION | $CO_2$ EQUIVALENT OF CHANGE OF LACTIC ACID | LACTIC ACID IN MGM. PER 100 CC. ARTERIAL BLOOD | $CO_2$ CAPACITY OF ARTERIAL BLOOD IN VOL. PER CENT | pH OF ARTERIAL BLOOD | MEAN BLOOD PRESSURE | OXIDATIVE ENERGY IN SMALL CALORIES PER KGM. HR. | NON-OXIDATIVE ENERGY IN SMALL CALORIES PER KGM. HR. | OXIDATIVE + NON-OXIDATIVE ENERGY IN SMALL CALORIES PER KGM. HR. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 |
| 1 | G. S. no. 1 | 10′ 8″ | 1.55 | 19.08 | 0.77 | 305.4 | 236.5 | 250 | −13 | | | | | 104 | 1,474 | | |
| 2 | A. S. no. 1 | 10′ 8″ | | | | | | | | | 59.7 | 43.57 | 7.39 | | | | |
| 3 | G. S. no. 2 | 19′ 8″ | 1.48 | 19.18 | 0.78 | 289.1 | 225.7 | 236.3 | −11 | −72 | | | | 92 | 1,395 | −88 | 1,307 |
| 4 | A. S. no. 2 | 19′ 5″ | | | | | | | | | 55.4 | 42.79 | 7.40 | | | | |
| 5 | G. S. no. 3 | 27′ 27″ | 1.52 | 19.20 | 0.82 | 281 | 229.8 | 230 | | −44 | | | | 86 | 1,356 | −54 | 1,302 |
| 6 | A. S. no. 3 | 27′ 21″ | | | | | | | | | 53.8 | 42.60 | 7.38 | | | | |
| 7 | Meth. blue 12 cc. m/10 dissolved in hot water | 31′ 39″ | | | | | | | | | c 52.9 | | | 76 | | | |
| 8 | G. S. no. 4 | 34′ 38″ | 2.30 | 18.20 | 0.79 | 444.4 | 349.9 | 364 | −14 | +34 | | | | 120 | 2,145 | +422 | 2,567 |
| 9 | G. S. no. 5 | 36′ 4″ | 2.60 | 17.79 | 0.77 | 511.8 | 395.8 | 419 | −23 | +346 | | | | 106 | 2,470 | +422 | 2,892 |
| 10 | A. S. no. 4 | 36′ 4″ | | | | | | | | | 63.1 | 40.12 | 7.34 | | | | |
| 11 | G. S. no. 6 | 38′ 20″ | 2.56 | 17.88 | 0.79 | 495.1 | 389.9 | 405 | −15 | −187 | | | | 68 | 2,390 | −227 | 2,163 |
| 12 | G. S. no. 7 | 47′ 45″ | 2.51 | 18.08 | 0.83 | 463.1 | 386.4 | 379 | +7 | −187 | | | | 60 | 2,234 | −227 | 2,007 |
| 13 | A. S. no. 5 | 47′ 53″ | | | | | | | | | 48.4 | 41.33 | 7.32 | | | | |
| 14 | G. S. no. 8 | 58′ 43″ | 2.62 | 17.95 | 0.83 | 484.9 | 403.5 | 397 | +7 | 0 | | | | 66 | 2,338 | 0 | |
| 15 | A. S. no. 6 | 58′ 45″ | | | | | | | | | 48.5 | 39.63 | 7.35 | | | | |
| 16 | m/10 meth. blue, 9 cc. | 65′ 1″ | | | | | | | | | c 48.6 | | | 64 | | | |

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 17 | G. S. no. 9 | 69′ 50″ | 2.74 | 17.91 | 0.87 | 488.5 | 422.7 | 400 | +23 | +110 | | | | 80 | 2,358 | +134 | 2,492 |
| 18 | G. S. no. 10 | 72′ 26″ | 2.59 | 18.13 | 0.89 | 451.3 | 399.6 | 369 | 30 | | | | | 53 | 2,177 | +134 | 2,311 |
| 19 | A. S. no. 7 | 72′ 39″ | | | | | | | | | 54.2 | 41.00 | 7.30 | | | | |
| 20 | G. S. no. 11 | 82′ 19″ | 2.46 | 18.39 | 0.94 | 405.6 | 379.8 | 331.8 | +48 | +117 | | | | 48 | 1,956 | +143 | 2,099 |
| 21 | A. S. no. 8 | 82′ 22″ | | | | | | | | | 61.8 | 37.78 | 7.25 | | | | |
| 22 | Lobeline (1 cc.) | 85′ 40″ | | | | | | | | | c 64.8 | | | 45 | | | |
| 23 | G. S. no. 12 | 89′ 8″ | 2.73 | 18.14 | 0.95 | 443.4 | 421.3 | 362.3 | +59 | 0 | | | | 42 | 2,138 | 0 | |
| 24 | G. S. no. 13 | 91′ 8″ | 2.78 | 18.14 | 0.97 | 441.5 | 429.6 | 361 | +69 | 0 | | | | 45 | 2,130 | 0 | |
| 25 | A. S. no. 9 | 91′ 8″ | | | | | | | | | 64.8 | 34.81 | 7.20 | | | | |
| 26 | G. S. no. 14 | 98′ 37″ | 2.10 | 18.79 | 0.95 | 340.1 | 322.8 | 278 | +44 | +150 | | | | 38 | 1,641 | 182 | 1,823 |
| 27 | G. S. no. 15 | 102′ 0″ | 1.88 | 19.07 | 0.98 | 294.9 | 288.6 | 236 | +53 | +150 | | | | 32 | 1,422 | 182 | 1,604 |
| 28 | A. S. no. 10 | 101′ 56″ | | | | | | | | | 75.6 | 31.54 | 7.22 | | | | |
| 29 | Drum stopped | 102′ 40″ | | | | | | | | | | | | | | | |

TABLE 6

*Experiment 6. Sodium sulphide and strychnine*

| OBSERVATION | PROCEDURE | TIME | PER CENT OF EXPIRED $CO_2$ | PER CENT OF EXPIRED $O_2$ | EXPIRATORY QUOTIENT | $O_2$ CONSUMPTION IN CC. PER KGM. HR. | $CO_2$ ELIMINATION IN CC. PER KGM. HR. | $CO_2$ ELIMINATION FROM OXIDATIONS BASED ON E.Q. IN CC. PER KGM. HR. | DIFFERENCE BETWEEN ACTUAL AND THEORETICAL $CO_2$ ELIMINATION | $CO_2$ EQUIVALENT OF CHANGE OF LACTIC ACID | LACTIC ACID IN MGM. PER 100 CC. ARTERIAL BLOOD | $CO_2$ CAPACITY OF ARTERIAL BLOOD IN VOL. PER CENT | pH of ARTERIAL BLOOD | MEAN BLOOD PRESSURE | OXIDATIVE ENERGY IN SMALL CALORIES PER KGM. HR. | NON-OXIDATIVE ENERGY IN SMALL CALORIES PER KGM. HR. | OXIDATIVE + NON-OXIDATIVE ENERGY IN SMALL CALORIES PER KGM. HR. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 |
| 1 | G. S. no. 1 | 9′ 19″ | 3.08 | 17.42 | 0.83 | 435.6 | 362.8 | 363 | | | | | | 136 | 2,110 | | |
| 2 | A. S. no. 1 | 9′ 57″ | | | | | | | | | 29.0 | 44.06 | 7.37 | | | | |
| 3 | G. S. no. 2 | 19′ 3″ | 3.14 | 17.28 | 0.82 | 453.1 | 369.7 | 378 | −8 | −36 | | | | 127 | 2,193 | | |
| 4 | A. S. no. 2 | 19′ 11″ | | | | | | | | | 26.8 | 43.77 | 7.39 | | | | |
| 5 | 45 cc. 0.033 m $Na_2S$ | 23′ 32″ | | | | | | | | | c 25.7 | | | 127 | | | |
| 6 | G. S. no. 3 | 28′ 59″ | 3.18 | 17.17 | 0.80 | 469.3 | 374.0 | 391 | −17 | 0 | | | | 135 | 2,272 | 0 | 2,272 |
| 7 | A. S. no. 3 | 29′ 19″ | | | | | | | | | 26.0 | 43.61 | 7.40 | | | | |
| 8 | G. S. no. 4 | 33′ 34″ | 3.06 | 17.27 | 0.79 | 457.3 | 359.6 | 381 | −21 | 0 | | | | 133 | 2,214 | 0 | 2,214 |
| 9 | G. S. no. 5 | 36′ 45″ | 3.05 | 17.31 | 0.79 | 452.7 | 358.6 | 377 | −18 | 0 | | | | 135 | 2,191 | 0 | 2,191 |
| 10 | A. S. no. 4 | 37′ 5″ | | | | | | | | | 26.0 | 42.36 | 7.39 | | | | |
| 11 | G. S. no. 6 | 47′ 12″ | 3.00 | 17.42 | 0.81 | 437.5 | 352.9 | 364.5 | −12 | 0 | | | | 126 | 2,118 | 0 | 2,118 |
| 12 | A. S. no. 5 | 47′ 27″ | | | | | | | | | 25.6 | 42.76 | 7.35 | | | | |
| 13 | G. S. no. 7 | 56′ 38″ | 2.83 | 17.65 | 0.82 | 408.0 | 332.8 | 340 | −7 | +16 | | | | 112 | 1,976 | +20 | 1,996 |
| 14 | A. S. no. 6 | 56′ 45″ | | | | | | | | | 26.6 | 41.96 | 7.35 | | | | |
| 15 | 10 cc. strychnine, 1 mgm. | 61′ 6″ | | | | | | | | | c 27.1 | | | 114 | | | |
| 16 | G. S. no. 8 | 65′ 10″ | 3.18 | 17.31 | 0.84 | 464.9 | 388.3 | 387.3 | +1 | +398 | | | | 107 | 2,250 | +484 | 2,734 |
| 17 | G. S. no. 9 | 68′ 12″ | 3.28 | 17.32 | 0.87 | 460.6 | 401.2 | 384 | +17 | +398 | | | | 103 | 2,230 | +484 | 2,714 |
| 18 | A. S. no. 7 | 68′ 16″ | | | | | | | | | 46.0 | 39.75 | 7.28 | | | | |

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 19 | G. S. no. 10 | 74′ 18″ | 3.32 | 17.47 | 0.93 | 436.9 | 407.1 | 364 | +43 | +251 | | | | 98 | 2,116 | +306 | 2,422 |
| 20 | G. S. no. 11 | 77′ 27″ | 3.38 | 17.47 | 0.95 | 433.3 | 413.5 | 361 | +52 | +251 | | | | 90 | 2,098 | +306 | 2,404 |
| 21 | A. S. no. 8 | 77′ 42″ | | | | | | | | | 61.8 | 35.70 | 7.21 | | | | |
| 22 | G. S. no. 12 | 87′ 42″ | 3.13 | 17.72 | 0.95 | 402.4 | 382.6 | 335.3 | +47 | +87 | | | | 83 | 1,948 | +105 | 2,053 |
| 23 | A. S. no. 9 | 87′ 14″ | | | | | | | | | 67.3 | 34.14 | 7.23 | | | | |
| 24 | G. S. no. 13 | 98′ 16″ | 2.99 | 17.78 | 0.92 | 398.3 | 364.9 | 332 | +33 | −33 | | | | 80 | 1,928 | −40 | 1,888 |
| 25 | A. S. no. 10 | 98′ 34″ | | | | | | | | | 64.8 | 35.59 | 7.23 | | | | |
| 26 | G. S. no. 14 | 107′ 45″ | 2.90 | 17.85 | 0.91 | 389.5 | 353.7 | 324.5 | +29 | −43 | | | | 90 | 1,885 | −52 | 1,833 |
| 27 | A. S. no. 11 | 108′ 0″ | | | | | | | | | 62.1 | 35.37 | 7.23 | | | | |
| 28 | Stopped drum | 109′ | | | | | | | | | | | | | | | |

If the removal is primarily non-oxidative, a decrease in the expiratory quotient is to be expected but inspection shows, with only one exception, that only minor changes occurred. Only in the last observation of figure 5 in which a lethal injection of methylene blue was administered was there a distinct change. The increase in this instance can most probably be attributed to the rapid formation of lactic acid associated with impaired oxidations. It will be noted that the changes in blood lactic acid and blood carbon dioxide capacity are by no means parallel.

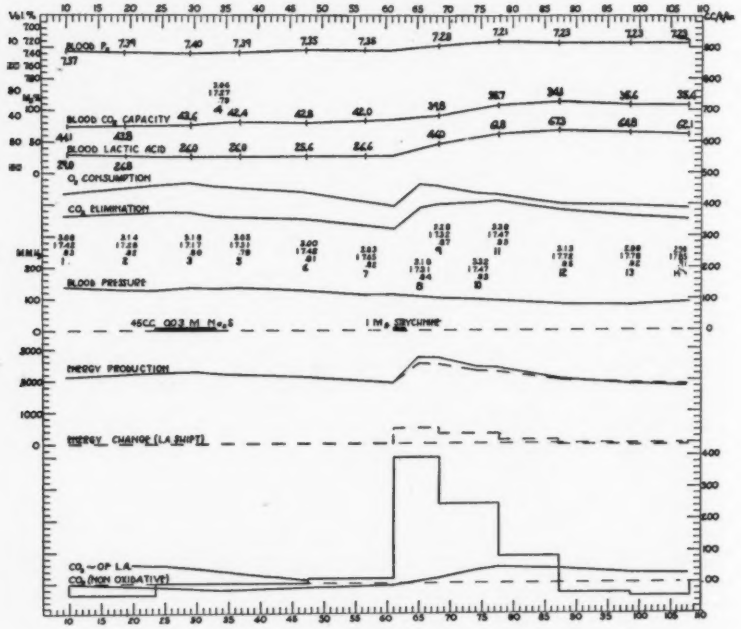



Fig. 6. Experiment 6

Since the increased acidity of the blood can be referred to the increased formation of carbon dioxide in the tissues, increased pulmonary ventilation is undoubtedly associated with both increased tissue acidity and increased tissue oxidations.

STRYCHNINE. Experiment 6 shows effects of intravenous injection of 1 mgm. of strychnine. The usual augmentation of respiratory movements occurred. These were associated with a marked increase in the hydrogen ion concentration of the blood followed by a partial recovery. There was a marked outpouring of lactic acid from the tissues followed by slower

resorption. The changes in blood carbon dioxide capacity of the blood ran parallel with those of blood lactic acid. Both carbon dioxide elimination and oxygen consumption showed a temporary increase associated with a rise in the expiratory quotient from 0.82 to 0.95 followed by a later decline to 0.92. This increase in the expiratory quotient is attributed primarily to the rapid formation of lactic acid. During the liberation of lactic acid the elimination of non-oxidative carbon dioxide is very small indicating that only 14.69 per cent of the lactic acid reacted with bicarbonate but during the more inclusive period from the beginning of injection up to gas sample 14 the carbon dioxide index mounts to approximately 0.30. The rapid rate of lactic acid production added to the increased oxygen consumption explains the marked increase in energy production. In view of the increased formation of carbon dioxide and lactic acid it is reasonable to assume that the tissues as well as the blood turned more acid during the period of augmented respiratory movements.

SODIUM SULPHIDE. Haggard (1921) and Haggard and Henderson (1922) have called attention to the strong stimulating action of intravenously injected sodium sulphide. According to Haggard, hydrogen sulphide is liberated in the blood stream and is responsible for the effects. After oxidation of the liberated hydrogen sulphide, which is rapid, stimulation ceases. Presumably the excitation, which Nack and Wulp (1929) have demonstrated to be of central origin, is referable to the reducing properties of hydrogen sulphide. Until further experiments are performed no comments will be made on the graph of experiment 6.

## SUMMARY

The effects of intravenous injection of ammonium chloride, methylene blue, strychnine and sodium sulphide are briefly described.

Intravenous injection of ammonium chloride increased the hydrogen ion concentration and decreased the carbon dioxide capacity of the blood. The initial effects on blood lactic acid were variable, the late effects were mostly an increase. The rate of gaseous exchange first increased, then decreased, then increased and returned towards normal. The changes in the expiratory quotient were of a minor order. The changes in magnitude of gaseous exchange were associated with similar variations in amplitude of respiratory movements.

Intravenous injection of methylene blue temporarily increased the hydrogen ion concentration of the blood and augmented gaseous exchange. A late decrease in blood lactic acid was frequently preceded by an initial increase. Despite these variations in blood lactic acid the changes in the expiratory quotient were small. The increase and decrease in hydrogen ion concentration and gaseous exchange were accompanied by parallel changes in volume flow of blood and respiratory movements.

Intravenous injection of strychnine increased the gaseous exchange, the blood lactic acid content and the hydrogen ion concentration of the blood. The blood carbon dioxide capacity was reduced and the expiratory quotient noticeably increased by the elimination of non-oxidative carbon dioxide. The increase in lactic acid liberated plus the increase in oxygen consumed resulted in a marked increase in the dissipation of energy. The metabolic changes produced by strychnine were accompanied by increased respiratory movements.

GENERAL REMARKS. Under the conditions of constant artificial ventilation supplied in this series of experiments it is reasonably certain that the increased respiratory movements accompanying lowered alveolar oxygen, cyanide injection, hemorrhage, the administration of methylene blue and strychnine are associated with increased cellular acidity throughout the body. However, it cannot be concluded from these experiments that increased acidity of the respiratory center would occur if the animal were allowed to control its ventilation, thereby lowering alveolar carbon dioxide pressure and augmenting elimination of carbon dioxide. Crucial experiments are still needed on this point. Experiments now in progress (Gesell, Brassfield, Krueger, Nicholson and Pelecovich, 1930) possibly suggest that when an animal is allowed to compensate for the effects of lack of oxygen by increased ventilation resting tissue (rectus femoris and testicle) may turn more alkaline than normal. If our assumptions are correct the calculated decrease in free carbon dioxide of these tissues appears to exceed the acid effect of decreased sodium bicarbonate as indicated by the lactic acid content of the muscle and testicle before and during oxygen lack. Whether these calculations can be applied to tissues exhibiting increasing activity and increased liberation ot volatile and fixed acid during oxygen lack, such as the respiratory center and the respiratory muscles, remains to be determined. For the present it is, therefore, difficult to evaluate the relative effects of changes in acid base equilibrium and oxidation-reduction equilibrium on pulmonary ventilation. To be sure increased respiratory movements may be associated with either decreased or increased rate of oxidations as evidenced in the present series of experiments. This might appear to minimize the importance of oxidations. On the other hand experiments of a different nature indicate independent effects of changes in acidity and changes in oxidation on tissue function. These results may ultimately be applicable to the subject of respiratory control. Glazer (1929), Winkler (1929), Gettel and Knoepp (1929), and Gay (1929) in a series of experiments have demonstrated that lowered alveolar oxygen and intravenous injection of cyanide increased the response of the muscle to direct stimulation, to stimulation through the motor nerve and to reflex stimulation through the afferent nerve fibers and that administration of carbon dioxide produced the opposite effects.

Eddy (1929) showed that the secretion of the submaxillary gland of pilocarpinised dogs was diminished by lowered alveolar oxygen and cyanide injection and increased by administration of carbon dioxide. Nicholson (1929) also obtained opposite effects of lowered alveolar oxygen and increased alveolar carbon dioxide on the outflow of cerebrospinal fluid. Carbon dioxide produced a definite augmentation whereas lowered alveolar oxygen almost invariably produced a decrease which was followed by an increase only after prolonged administration of low oxygen mixtures. Gesell and Nyboer (1929) found that lowered alveolar oxygen accelerated cardiac rhythm and that carbon dioxide slowed it. The difference in effects of administration of gaseous mixtures of low oxygen pressure and high carbon dioxide pressure in these various types of experiments is easily demonstrable. Only when we come to pulmonary ventilation are the effects more comparable and more inseparable. Even here there are differences as was pointed out by Haldane (1922) and others (see Gesell, 1929 Ergebnisse der Physiologie). Lowered alveolar oxygen tends to increase pulmonary ventilation largely by accelerated rhythm and carbon dioxide increases ventilation largely by augmented breath volume. Gesell and Nyboer (1929) have called attention to a similarity of changes in cardiac and respiratory rhythm under various conditions. They have noted that both cardiac and respiratory rhythm are always definitely accelerated by lowered alveolar oxygen and that not infrequently both may be slowed by increased alveolar carbon dioxide pressure. On the basis of a comparable response it has been proposed that there may be a common mechanism of rhythmic discharge in the respiratory and cardiac rhythm centers. It is hoped that the following series of experiments on the correlation of numerous respiratory factors, in which the animals will be allowed to control their ventilation, will lead to a clearer differential evaluation of the effects of impaired oxidations and increased hydrogen ion concentration in the regulation of respiration.

## BIBLIOGRAPHY


Glazer. 1929. This Journal, lxxxviii, 562.
Winkler. 1929. This Journal, lxxxix, 243.
Gettel and Knoepp. 1929. Proc. Soc. Exp. Biol. and Med., xxvi, 830.
Gay. 1929. Proc. Soc. Exp. Biol. and Med., xxvi, 825, 826.
Eddy. 1929. This Journal, lxxxviii, 534.
Gesell and Nyboer. 1929. Proc. Soc. Exp. Biol. and Med., xxvi, 834.
Haldane. 1922. Respiration. Yale Press, New Haven.
1921. Journ. Physiol., lv, 265.
Koehler. 1927. Journ. Biol. Chem., lxxii, 99.
Salkowski. 1878. Zeitschr. f. Physiol. Chem., i, 1.
Heymans. 1923. Arch. int. de. Pharmacod. and Therap., xxvii, 319.
Gesell. 1929. Ergebn. d. Physiol., xxviii, 346.
Haggard. 1921. Journ. Biol. Chem., xcix, 519.
Haggard and Henderson. 1922. This Journal, lxi, 289.
Nack and Wulp. 1929. This Journal, lxxxix, 315.
Nicholson. 1929. Proc. Soc. Exp. Biol. and Med., xxvi, 831.

# CONDUCTION IN DIFFERENT PARTS OF THE SMALL INTESTINE

## I. Distance Traversed by a Disturbance and Rate of Travel

WALTER C. ALVAREZ AND KIYOSHI HOSOI

With the technical assistance of Mildred Foster and E. C. Woolley

*From the Division of Medicine, The Mayo Clinic, Rochester, Minn.*

Received for publication March 21, 1930

To a large extent advance in the science of gastro-enterology waits today on more knowledge in regard to the mode of conduction of waves and stimuli along the digestive tract. Perhaps the most important point established thus far is that disturbances set up by stimulation of the small bowel travel farther caudad than orad. This was noticed by Bayliss and Starling during the course of their classic experiments; it was later amply confirmed by Alvarez and Starkweather, and it has since been commented on by Ganter, Pearcy and van Liere, Gelhorn and Weidling, and Nolf.

During the last few years we have been studying some of the details of conduction in the small bowel of the rabbit. In experiments on the intact gut the animals were anesthetized with urethane, the spinal cord was pithed distal to the fourth dorsal vertebra, and the abdomen was opened in a large tank of warm saline solution. By using commercial sodium chloride we obtained cheaply and quickly a solution sufficiently balanced for our purposes.[1] Excised bowel was studied in a Pyrex bread-baking dish filled with warm aerated Locke's solution.

In order to see how far the effect of an electric stimulus would be transmitted in different regions of the bowel we fastened two of the clamp electrodes, described elsewhere (Alvarez and Hosoi, 1929a), to the ends of a loop which was usually 15 cm. long. Sometimes we used longer loops but the shortness of the mesenteric fan often hampered us. Threads running to light recording levers were fastened with the help of small wire clips to the peritoneal coat of the bowel opposite the points gripped by the electrodes so that when stimuli were thrown in the resulting local contraction would be recorded. The passage of propagated disturbances along the length of the loop between the two electrodes was recorded with the help of other threads fastened here and there, usually at intervals of 5 cm. All

[1] We have recently found that some brands of salt are toxic and cannot be used.

the threads were fastened in such a way that the contractions of the circular muscle were the ones recorded. By stimulating at the orad end of the loop we could study conduction caudad and by stimulating at the caudad end we could study conduction orad.

The stimulus used was generally a faradic tetanizing one. A Harvard inductorium was used with one dry cell in the primary circuit and the secondary coil at 4 cm. Single break faradic shocks sometimes produced a weak contraction at the point stimulated but often there was no response and there never was any sign of conduction of wavelets along the loop. A constant galvanic current of from 5 to 20 milliamperes produced contractions with a short latent period and an amplitude smaller than that of the responses to faradic stimulation. The disturbances so produced did not spread far in either direction. For a time we thought that they did not spread at all but when we placed recording levers close to the point stimulated we found that wavelets could commonly be detected 2 or 3 cm. distant and occasionally 5 or 6 cm. distant. Galvanic currents, interrupted six times in a second, produced disturbances which spread orad and caudad much as did those produced by faradic stimulation. Faster interruption of the galvanic current sometimes produced a traveling disturbance but often it did not. The possible significance of these differences in response to different currents will be discussed in a later paper.

THE DISTANCE TO WHICH THE DISTURBANCE PRODUCED BY FARADIC STIMULATION TRAVELED. Before we proceed to an analysis of the records we must first warn the reader that distances measured along the bowel of the rabbit never can be anything but rough guesses. With a little pull one can double the distance between two markers, or the distance may double of itself when a full and active loop empties and loses its tone. Hence, when in this paper we say 5 cm. or 15 cm. we mean a distance which was about that at the beginning of the experiment.

We must admit also that we often have had difficulty in distinguishing between a rise in the record due to conduction of a stimulus and one due to some local spontaneous shortening of the muscle. For this reason we discarded the many doubtful records and studied the better ones statistically in the hope that distribution polygons and their modes would, in spite of the inclusion of some falsely interpreted records, give fairly trustworthy information.

Occasionally the lever descends before it rises, either at the point stimulated or a few centimeters orad or caudad, and in such cases the interval of time between the stimulus and the beginning of inhibition appears to be the same as it usually is between the stimulus and the first upward movement of the lever.

Figure 1 shows, in the form of percentage distribution polygons, the frequency with which, at certain distances orad and caudad from a point

stimulated faradically, a disturbance in the activity of the bowel could be detected. The shapes of these polygons would doubtless be somewhat altered by further work because much depends on the relative numbers of animals encountered with good or poor conduction. It is probable also that if, more often, we had used long loops, a few of the waves that were found to travel 15 cm. could have been detected 20 cm. away from the point stimulated.

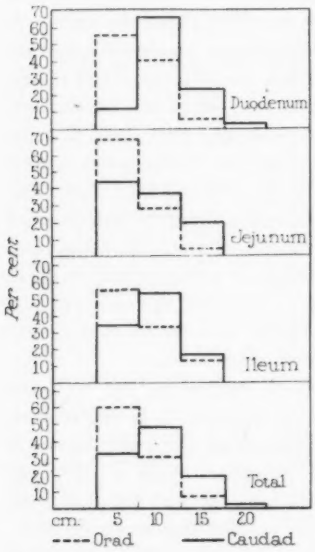



Fig. 1. Percentage distributions showing the frequency with which a disturbance in the activity of the bowel could be detected at certain distances orad and caudad from a point stimulated faradically. There were 477 observations on bowels from forty-two rabbits.

Another defect of these polygons is that they do not show how large the number of experiments was in which, after a stimulus, wavelets could not be detected even 5 cm. away. In the course of 672 stimulations in twenty-five normal animals, wavelets, or what for the sake of convenience we shall call wavelets, were detected at a distance of 5 cm., in the duodenum, in 28.2 per cent of the experiments, in the jejunum in 35.7 per cent, in the upper part of the ileum in 27.2 per cent, and at the lower end of the ileum in 19.4 per cent.

The polygons represent 477 observations made in forty-two animals. They show first that the range is from 1 to 20 cm. caudad and from 1 to 15 cm. orad. Conduction over the longer distances was observed most frequently in the duodenum. It was observed in a few instances in other parts of the bowel when, in another series of experiments, we placed the first recording thread 20 cm. from the point stimulated.

The modal or commonest distance traversed by wavelets orad was 5 cm. while the modal distance for wavelets traveling caudad was 10 cm. It is not easy to study conduction in the duodenum because this part of the bowel is very active; there are many spontaneous changes in tone which make it hard to interpret the records, and the irritability is so great that many of the stimuli give rise to peristaltic rushes. Since the rushes behave differently from the wavelets we discarded all those records in which the traveling disturbances showed no sign of a decrement.

*Long distance conduction.* When we found that we could so rarely de-

tect signs of conduction more than 15 cm. away from a point stimulated we suspected that Alvarez and Starkweather were mistaken when they thought they could detect signs of conduction 40 cm. or more caudad to the point stimulated, but later it occurred to us that they might have been dealing with another type of conduction which is probably mediated by long nerve paths either in the mesentery or in the wall of the bowel. There is no question that activities in one part of the bowel can almost immediately affect the tone and rhythmic activity of other parts. As Alvarez and Mahoney pointed out, a rush wave starting in the duodenum will often cause the lower end of the ileum to become tonic and abnormally active, and the emptying of the cecum or rectum often will cause temporary inhibition of the waves of the stomach or of the rhythmic movements of the duodenum.

We therefore changed our technic; we fastened the first recording levers 30 and 40 cm. caudad to the point stimulated, and then we occasionally found small but definite increases in the amplitude of the rhythmic contractions. This response came so quickly that conduction must have taken place by way of efficient nerves. We could demonstrate this type of conduction in only a few animals, but it may well be that another type of stimulus, perhaps from the inner surface of the bowel, would have been more effective.

*Absence of descending inhibition.* The disturbance spreading out from a point stimulated almost always showed itself as a rise in the level of the record. In hundreds of experiments we rarely saw anything that resembled a myenteric reflex; that is, contraction orad and relaxation caudad. As already mentioned we sometimes saw, either at the point stimulated or a short distance away, what looked like inhibition followed by contraction. Sometimes this was due to the slipping of the electrode off the bowel and onto the mesentery where it probably stimulated postganglionic inhibitory nerves (fig. 2[a]).

As one would expect from the work of Bayliss and Starling who demonstrated their "Law" only in denervated bowel, we occasionally were able to show descending inhibition in animals in which the vagus and splanchnic nerves had been cut and allowed to degenerate; but even in these animals, in perhaps 98 per cent of the experiments, the response, if any, was contraction at the point stimulated and also orad and caudad.

Occasionally, as can be seen in figure 2[b], after traveling for several centimeters as a wave of excitation, the disturbance produced by the electric stimulus caused temporary relaxation of the muscle or a lowering of the amplitude of a few rhythmic contractions. One wonders whether the phenomenon is due to a weakening of the stimulus or the fact that nerve tracts that mediate inhibition are longer than those that mediate stimulation. The problem is complicated by the fact that rarely (fig. 2[c]) depression in one segment was followed by contraction in the segments imme-

diately caudad, or no contraction in one or two segments was followed by contraction in the next segment (figs. 2[d] and 2[e]).

*The nature of the traveling disturbance.* Throughout this discussion we have used the term wavelet although we doubt if the disturbance which spreads orad and caudad from a point stimulated can always be identified

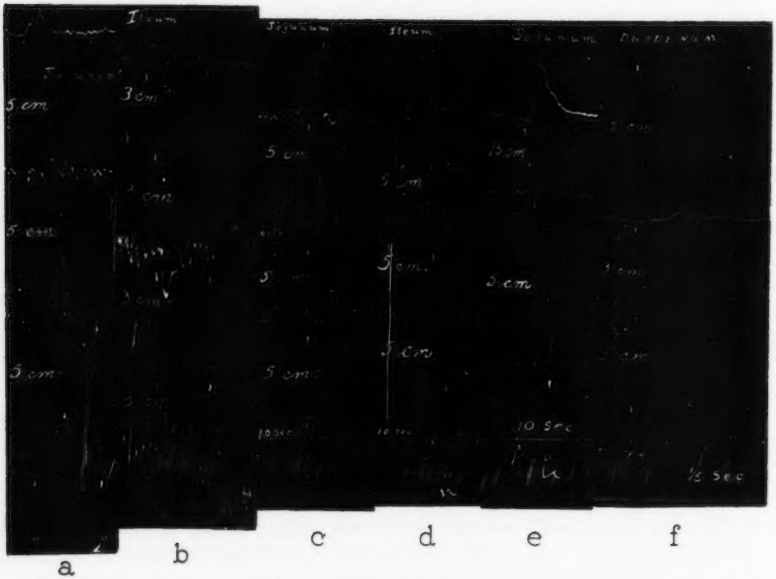



Fig. 2. Records from intact bowel stimulated with faradic current. The figures between the lines represent distances on the bowel between recorders. Synchronous points are marked. *a.* Probably a rush following stimulation at the orad end. Following this, stimulation at the caudad end produced local inhibition, and contraction 5 cm. orad. *b.* Stimulation at the orad end produced a traveling disturbance which showed itself first as a contraction, then relaxation, and later contraction again. Stimulation at the caudad end produced only contraction. *c.* Stimulation orad produced inhibition 5 cm. caudad and contraction 10 cm. caudad. *d.* Stimulation at the orad end had little or no effect for a distance of 10 cm. and then produced contraction. *e.* Stimulation at the orad end had practically no effect at a point 15 cm. caudad but produced contraction at a point 20 cm. caudad. *f.* Faradic stimulation at the caudad end produced locally slight contraction followed by relaxation. At a point 3 cm. orad it produced contraction.

as a wave. As we watched we could sometimes see a contraction traveling over the first five centimeters of bowel near the electrode but beyond this the disturbance was generally too faint to be detected with the unaided eye.

*Relation between strength of stimulus and distance traversed.* With weak

stimuli there appeared to be some relation between the distance to which a wavelet traveled and the height of the initial contraction, but with strong stimuli there was little sign of any such relation.

The bowel was capable of much better conduction in some animals than in others, but why this was we could not learn. In one rabbit which presented the picture of unusually frequent and rapid peristaltic rushes, conduction as tested by faradic stimulation was not particularly good, nor was it particularly good in another rabbit with diarrhea and an unusully active cecum. Similar individual differences were found in the efficiency of conduction when the interrupted galvanic current was used.

*Rate of travel of the disturbance produced by faradic stimulation.* Again we must warn the reader that the measurements presented can be only approximate, and that some erroneous ones doubtless have been used in calculating averages. Especially when one is analyzing short records on a fast drum it is hard to be sure that a rise in the lever was due to the electric stimulus and not to some spontaneous activity. All we could do was to discard doubtful records and then study the remainder statistically in the hope that positive errors would balance negative ones and that modes and means would give information of value. The fact that when mean rates of conduction orad and caudad in different parts of the bowel were calculated they were found to fall into graded sequences (fig. 4), encouraged us to hope that we had obtained figures that mean something.

One difficulty which we had to overcome before we could get concordant results arose from the fact that a sudden marked lengthening of the latent period of contraction at the point stimulated had no effect on the time of arrival of the wavelet 5 cm. away. Let us say that in a certain experiment the bowel at the point of stimulation responded to several successive stimuli after a latent period of 0.2 second. Each time the bowel 5 cm. away contracted 1.2 second after the stimulus, showing that the rate of conduction was 5 cm. a second. Then the latent period at the point of stimulus suddenly lengthened to 0.8 second. If the disturbance detected 5 cm. away were a wave of contraction beginning in the muscle at the point stimulated it should have taken 1.8 second to reach the next recorder but actually it arrived as before, after 1.2 second. This observation agrees with a number of others, all of which indicate that the disturbance spreads out along conducting paths in Auerbach's plexus independently of anything that may be happening in the muscle fibers in the segment stimulated (figs. $2^a$ and $2^f$). For this reason, when computing rates of conduction we found it essential to subtract from the interval between the stimulus and the rise of the lever, not the actual latent period observed at the point stimulated, but the usual one of 0.2 second. It is curious also that the time which the stimulus required to traverse the first 5 cm. of bowel was generally much longer than that required to traverse the second 5 cm.

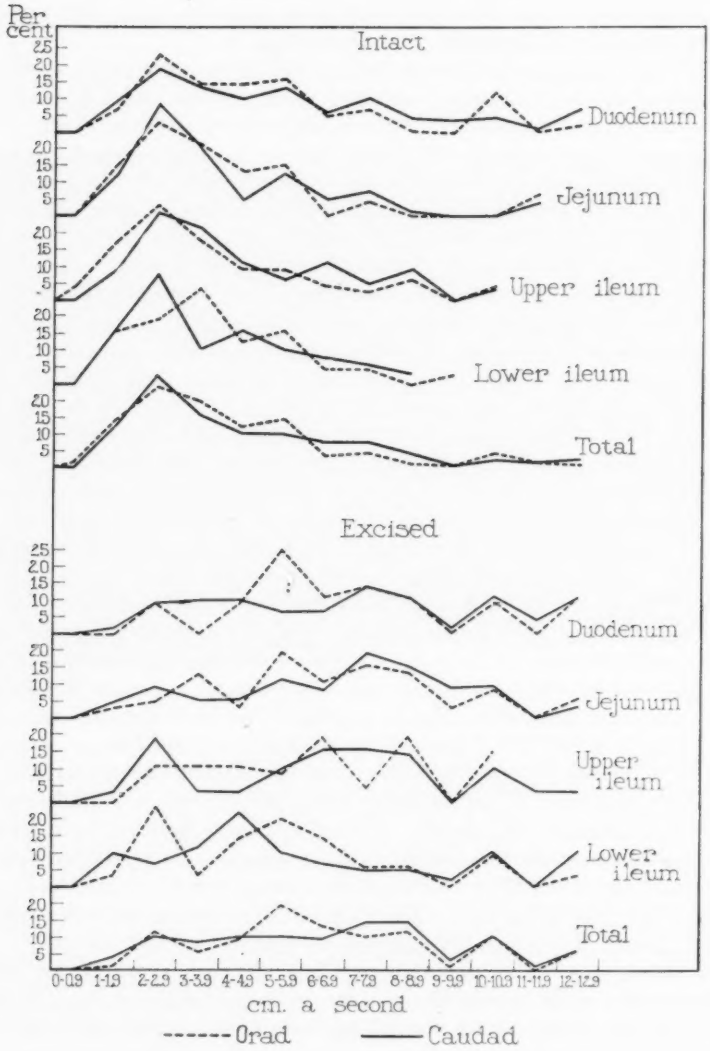



**Fig. 3. Percentage distributions showing the frequency with which conduction at different rates was observed in intact and in excised bowel. There were 475 measurements on bowels from thirty rabbits.**

For the benefit of those who later may wish to repeat this work we will say that success depends to a large extent on the speed with which the electrodes are attached and the measurements made. Speed is particularly essential when one is working with excised bowel because after a time the responsiveness to stimuli and the ability to conduct disappear. As Nolf has pointed out, if the excised bowel is to retain its properties of conduction, the Locke's solution bathing it must at all times be kept well oxygenated, and when the bowel is not being studied, it must be kept so cold that metabolic processes are practically stopped. Ascanio and Alvarez have shown that if the surviving bowel is to be kept in good condition for several hours it either must be functioning actively with a plentiful supply of oxygen or else it must not function at all. Metabolism with deficient oxygenation leads rapidly to autolysis and death.

Figure 3 shows the distribution of 475 measurements made in four different parts of the intact bowels of thirty apparently normal rabbits. In every instance the length of the loop was 5 cm. Conduction caudad was observed 286 times and conduction orad 189 times. The range was from 1 to 13 cm. a second and there was a poorly defined mode at the point corresponding to 2.5 cm. a second. Figure 4 shows that the mean rate of conduction was fastest in the duodenum and slowest in the lower end of the ileum. Ordinarily one could not put trust in means with such large probable errors but, as will be noted in figure 3, the whole distribution curve representing data from the lower end of the ileum is so much shorter than that for the duodenum that there can be little doubt about the difference in the means.

The slow rate of conduction found in the ileum fits with a number of other observations which have been described in recent papers by Alvarez et al., which indicate that the lower end of the small bowel is poorly supplied with myenteric nerves. The rate of travel of the wavelets was about the same as that of beginning peristaltic rushes.

A study of the means suggests that conduction caudad was a little faster than conduction orad. This is what one would expect from the gradient theory of peristalsis, but the measurements were often so hard to make, the differences orad and caudad were so slight (one would expect them to be so with a measured distance of only 5 cm.), and the probable errors so large that we cannot be sure of anything. Under the circumstances it is remarkable that the expected difference in rates orad and caudad is to be found in almost every group of measurements made in the four different parts of the bowel, intact and excised, and in both normal and vagotomized animals. Taking together all the measurements made in all parts of the bowel of the intact normal animal, the means for the rates orad and caudad were respectively 4.15 and 4.50 cm. a second. The difference of $0.35 \pm 0.16$ cm. a second is only 2.2 times its probable error and this gives

a probability of only 6.3:1 that it is significant. According to the Chi-square test (Pearl) the probability is 5.2:1 that the difference between the two distributions is not due to random sampling.

CONDUCTION IN EXCISED BOWEL. Conduction was more easily demonstrated in the excised bowel than in the intact one, which suggests that in the living animal the extrinsic nerves maintain an inhibitory effect. That this inhibition is exerted mainly by the vagus nerves was shown by the fact that when they were cut and allowed to degenerate, conduction was as easy to demonstrate in the intact bowel as in the excised. Furthermore, inhibition having been removed by vagotomy, we could hardly expect to see further improvement in conduction after excision of the bowel, and actually none was observed.

Degenerative section of the major splanchnic nerves not only removed inhibition, but apparently led to the injury of some of the paths along which conduction normally takes place. As a result, in splanchnicotomized animals conduction over a distance of 5 cm. was seldom demonstrable. This offers perhaps some confirmation of the recent work of van Campenhout who, after excising a part of the neural crest of frog and chick embryos and allowing them to develop, became convinced that the myenteric plexus of the small bowel is formed from sympathetic cells, and not from vagal cells, as has previously been taught.

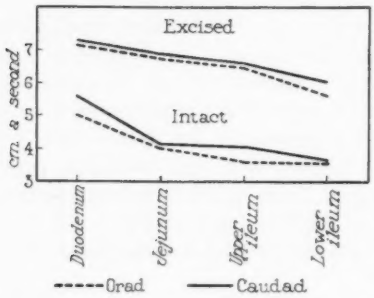



Fig. 4. The gradation in mean rate of conduction from duodenum to ileum with intact and excised bowel.

Table 4 in an article by Alvarez and Hosoi, gives for the different types of experiment performed, the number of instances in which conduction was demonstrable, expressed as percentages of the number of stimuli thrown in. As stated in the introduction to this paper, in the intact animal the electrodes with recording levers were placed 5 cm. apart on the bowel. In the experiments on excised bowel the electrodes were placed 10 cm. apart and another lever was fastened midway between them so that we could take note not only of a number of wavelets traveling 5 cm. but also of the number of those few that traveled 10 cm.

Figure 3 shows the distributions of the rates of conduction observed in the excised bowel. It will be seen that these rates tended to be faster than those observed in the intact bowel. The range is greater and the distribution curves show no decided mode. This absence of a mode is due largely to the fact that bowel from different animals varied so markedly in its

ability to conduct that the range for one animal sometimes barely overlapped the range for another. Figure 4 shows that the means ranged from 7.2 cm. a second in the duodenum to about 6.0 cm. a second in the lower part of the ileum. Again, there was a gradation from duodenum to ileum, and the rate of conduction caudad was a little faster than that of conduction orad. This is curious in view of the fact that the differences are too small to be statistically significant.

COMMENT. A number of the observations made during the course of this work indicates that Nolf is right and that conduction along the bowel is mediated by nerves. These nerves are apparently stimulated by a suitably interrupted current but not by a constant one, and the same appears to be true of the extrinsic nerves of the digestive tract. Judging from the very slow rate of conduction observed, the nerves in the myenteric plexus must be sluggish, much like those found in lowly forms of life. Such sluggishness might explain their failure to respond well to single shocks.

More evidence of a nervous type of conduction is to be found in the fact that the time of appearance of a contraction 5 cm. distant from the point stimulated appears to be independent of what happens at this point. In fact, in many instance the lever attached 5 cm. orad to the point stimulated was observed to rise a few seconds before the one attached to the segment in the grip of the electrode. Comment on this and many other curious observations made by us during the course of this study will be reserved for another paper.

## SUMMARY

The small intestine of rabbits was stimulated with a faradic tetanizing current and records were obtained of the local contraction and of the disturbance which spread orad and caudad.

Make and break faradic and galvanic stimuli produced a local contraction but little or no sign of a traveling disturbance. Interrupted galvanic stimulation produced effects similar to those of interrupted faradic stimulation. After faradic stimulation a disturbance traveled orad from 1 to 15 cm. and caudad from 1 to 20 cm. The modal distance orad was 5 cm. and the modal distance caudad was 10 cm. The best conduction was seen in the duodenum.

In a few animals a disturbance could be detected 40 or 50 cm. caudad to the point stimulated. Such conduction was rapid and was mediated probably by nerves in the mesentery.

The response to electric stimulation of the rabbit's bowel is practically always contraction locally and orad and caudad. Bayliss and Starling's law does not hold true for the rabbit.

Occasionally after traveling a short distance as a wave of contraction

the disturbance produced by an electric stimulus manifested itself as a wave of relaxation.

In the intact bowel the rate of conduction varied from 1 to 13 cm. a second with a mode about 2.5 cm. The mean varied from 5 cm. a second in the duodenum to 3.8 cm. a second in the terminal ileum. In excised bowel the mean rate varied from 7.2 cm. a second in the duodenum to 6.0 cm. a second in the terminal ileum. In both intact and excised gut the rate caudad was a little faster than the rate orad.

Conduction in the wall of the bowel appears to take place by way of nerves which are sluggish like those in lowly forms of life.

## BIBLIOGRAPHY


ALVAREZ, W. C. AND K. HOSOI. 1929a. This Journal, lxxxix, 182
1929b. This Journal, lxxxix, 187.

ALVAREZ, W. C. AND L. MAHONEY. 1924. This Journal, lxix, 211.

ALVAREZ, W. C. AND E. STARKWEATHER. 1919. This Journal, l, 252.

ALVAREZ, W. C., K. HOSOI, A. OVERGARD AND H. ASCANIO. 1929. This Journal, xc, 631.

ASCANIO, H. AND W. C. ALVAREZ. 1929. This Journal, xc, 611.

BAYLISS, W. M. AND E. H. STARLING. 1899. Jour. Physiol., xxiv, 99.

GANTER, G. 1923. Pflüger's Arch., cci, 101.

GELLHORN, E. AND K. WEIDLING. 1926. Pflüger's Arch., ccxv, 219.

NOLF, P. 1928. Arch. internat. de physiol., xxx, 317.

PEARCY, J. F. AND E. J. VAN LIERE. 1926. This Journal, lxxxviii, 64.

PEARL, R. 1923. Medical biometry and statistics. Philadelphia, W. B. Saunders Co., 379 pp.

VAN CAMPENHOUT, E. 1929. Proc. Soc. Exper. Biol. and Med., xxvi, 824.

# OXYGEN UTILIZATION IN THE LEGS OF NORMAL MEN

## I. Effect of Posture

M. FLORKIN,[1] H. T. EDWARDS and D. B. DILL

*From the Fatigue Laboratory, Morgan Hall, Harvard University*

Received for publication March 26, 1930

There are two direct methods of studying the effects of posture upon the circulation of blood in the legs. Bock, Dill and Edwards (1930) employed one of these when they injected histamine into an ankle vein and found that the time elapsed before the heart rate accelerated was about twice as long in the erect as in the recumbent posture.[2] This indicates that the velocity is decreased when standing but does not prove that the quantity of blood flowing through the legs in unit time is less, because the cross-sectional area of the path may be increased enough to maintain a constant volume-flow.

The other method involves puncture of the femoral vein and determination of oxygen utilization in the legs.[3] Blood thus obtained in contrast to that from a superficial vein, represents the metabolic processes of a considerable fraction of the skeletal muscles and possibly as much as 30 per cent of the body volume. Blalock (1929) has made a study of patients with varicose veins and includes the record of one subject with normal veins. In this one subject he found a considerable decrease in oxygen content with change from the recumbent to the erect posture. Related investigations are reviewed in his paper and need not be referred to here. Our experiments have been carried out on 10 normal subjects with the aim of determining 1, the extent of variation among individuals in respect to oxygen utilization in skeletal muscles at rest, and 2, the effect of posture on oxygen utilization.

[1] Fellow of the C. R. B., Educational Foundation.

[2] This is a technique developed by Weiss and his associates. Apparently Hering was the first to develop a method for estimating "least time" of blood flow. Poiseuille (Compt. rend., 1843, xvi, 60) applied the method to the horse by injecting potassium ferricyanide solution in one jugular and determining the time of arrival in the other by analysis of a series of blood samples. Values for "least time" ranged from 25 to 34 seconds.

[3] We are indebted to Drs. Soma Weiss and L. B. Ellis of the Boston City Hospital for having taught us the technique of puncturing the femoral vein.

TABLE I

| SUBJECT* | | $HbO_2$ CAPACITY | $HbO_2$ CONTENT | | OXYGEN TRANSPORT | PULSE RATE |
|---|---|---|---|---|---|---|
| | | *vols. per cent* | *vols. per cent* | *per cent saturation* | *vols. per. cent* | |
| Doorakian | Lying | 19.54 | 17.58 | 90.2 | 0.98 | 98 |
| | Standing | 19.73 | 12.92 | 65.4 | 5.81 | 75 |
| | Δ | +0.19 | −4.66 | −24.8 | +4.83 | −23 |
| Stevens | L | 20.05 | 16.20 | 80.8 | 2.85 | |
| | S | 21.25 | 11.62 | 54.7 | 8.57 | |
| | Δ | +1.20 | −4.58 | −26.1 | +5.72 | |
| Dill | L | 18.17 | 13.84 | 76.1 | 3.42 | 56 |
| | S | 19.59 | 8.44 | 43.1 | 10.17 | 60 |
| | Δ | +1.42 | −5.40 | −33.0 | +6.75 | +4 |
| Anderson | L | 21.72 | 18.26 | 84.2 | 2.37 | 72 |
| | S | 22.87 | 12.64 | 55.3 | 9.09 | 80 |
| | Δ | +1.15 | −5.62 | −28.9 | +6.72 | +8 |
| Jordan | L | 20.07 | 15.50 | 77.2 | 3.57 | 62 |
| | S | 20.80 | 6.42 | 30.8 | 13.34 | 75 |
| | Δ | +0.73 | −9.08 | −46.4 | +9.77 | +13 |
| Koerper | L | 21.17 | 15.83 | 74.8 | 4.28 | 72 |
| | S | 23.07 | 8.76 | 37.9 | 13.16 | 76 |
| | Δ | +1.90 | −7.07 | −36.9 | +8.88 | +4 |
| Bowen | L | 20.50 | 13.34 | 65.0 | 6.14 | 79 |
| | S | 21.82 | 7.70 | 35.3 | 13.03 | 88 |
| | Δ | +1.32 | −5.64 | −29.7 | +6.89 | +9 |
| Consolazio | L | 19.83 | 13.59 | 68.5 | 5.25 | |
| | S | 20.46 | 7.52 | 36.8 | 11.92 | |
| | Δ | +0.63 | −6.07 | −31.7 | +6.67 | |
| Smith* | L | 15.36 | 10.17 | 66.3 | 4.42 | 72 |
| | S | 16.95 | 5.36 | 31.7 | 10.74 | 132 |
| | Δ | +1.59 | −4.81 | −34.6 | +6.32 | +60 |
| Bock | L | 20.96 | 11.86 | 56.6 | 8.05 | 66 |
| | S | 22.90 | 7.31 | 31.9 | 14.41 | 70 |
| | Δ | +1.94 | −4.55 | −24.7 | +6.36 | +4 |
| Average | L | 19.74 | 14.62 | 74.0 | 4.13 | |
| | S | 20.95 | 8.87 | 42.3 | 11.02 | |
| | Δ | +1.21 | −5.75 | −31.7 | +6.89 | |

* Smith was a hospital patient with gastric ulcer but with a normal heart. Aside from Bock, Dill and Smith, the subjects were college students ranging in age from 19 to 26 years.

The subjects came to the laboratory without breakfast and rested on a bed for 30 to 50 minutes. The puncture was made under novocaine anesthesia and was without pain or unpleasant after-effects. Then the subject stood quietly and after 10 to 15 minutes the second puncture was made while the subject continued standing. About 20 cc. of blood were drawn into a well-oiled syringe and transferred under oil to a tube containing heparin. After mixing, 5 cc. were immediately transferred to a small sampling tube over mercury and held at 5° until analyzed. A similar portion was saturated with oxygen by equilibration with air at 20° for 30 minutes in a tonometer of about 300 cc. capacity. The oxygen content was then determined in duplicate by Van Slyke's procedure and the usual corrections were made for free oxygen.

The results are shown in the table. When the subjects were reclining, saturation of venous blood with oxygen ranged from 56.6 to 90.2 per cent. In the standing posture the range in saturation was from 30.8 to 65.4. Since arterial blood may be taken as 95 per cent saturated, it is easy to calculate oxygen transport, that is, the volume of oxygen transported per 100 volumes of blood. Thus the range in oxygen transport varies from 0.98 to 8.05 vols. per cent in reclining subjects and from 5.81 to 14.41 in standing subjects. The average oxygen transport while standing was 2.7 times greater than the average value for reclining subjects.

Due to the increase in metabolism with change from the recumbent to the erect posture it is not possible to estimate exactly the change in volume-flow of blood in the legs. Benedict and Benedict (1924) have found that such a postural change produces an increase in total metabolism of about 10 per cent. Not all of this increase is in the legs but the assumption that the tissues drained by the femoral vein have an increase of one-third in metabolic rate probably is liberal. This means, if there were an unchanged rate of blood flow, with change from reclining to erect posture, there would be an increase in the oxygen transport from 4.13 to 5.5 vols. per cent. Since the observed value for oxygen transport in fact increased to double the latter quantity or 11.0 vols. per cent, it appears that in the erect posture one-half as much blood flows through the legs in unit time as in the reclining posture.

These results have many interesting implications. Since Bock, Dill and Edwards (1930) have found that the decrease in velocity of venous return in the legs upon change from the reclining to the erect posture is of the same order of magnitude as our observations indicate for change in volume flow, it appears that the mean path is not much changed with postural change. Superificial veins of the legs obviously contain more blood when a subject is standing but the approximate correspondence of velocity and volume flow suggests that the deeper veins are less subject to change in diameter with change in posture. Yet it is a common observa-

tion that the volume of the legs often increases while an erect posture is maintained. It seems likely that the increase in leg volume is due only in part to blood stagnation, for this implies an increase in cross-sectional area of the mean path. A large part of the increase probably is due to transfer of protein-free fluid from blood to tissues as indicated by the observations of Thompson, Thompson and Dailey (1928). In harmony with their results we have found that change in oxygen capacity is associated with a corresponding change in serum protein concentration as indicated by the refractive index.

The well-defined effect of postural change on oxygen transport is no less interesting than the fact that individual values extend from 1 to 8 volumes per cent in reclining subjects. Doorakian was the only subject who exhibited excitement during the first puncture. Others may have been emotionally disturbed but none admitted it. The situation was different when the subjects were erect. Koerper fainted outright but waited two hours and was then able to proceed without concern. Bowen, Smith and Jordan were somewhat upset, Consolazio and Dill were slightly affected and the others appeared to be wholly unconcerned.

Such variations in rate of flow through the legs are almost certainly not accompanied by corresponding changes in cardiac output. Thus Grollman (1929) has found that emotional disturbances have little effect on cardiac output, the maximum increase being one-fourth. It appears, therefore, that there may be reciprocal changes in rate of flow in different parts of the body. If the heart is putting out blood at a constant rate, changes in rate of flow through the periphery must be balanced by reciprocal changes elsewhere. This is true not only of variations in individuals while reclining but also of the average change in rate with change in posture. No one has found that postural change is accompanied by a change in cardiac output of the order corresponding to the change we have observed in flow through the legs. Accordingly when one changes from the reclining to the erect posture, it is possible that there is an increased rate of flow in the central area.

The extent of variation in blood flow through the legs at rest indicates a corresponding variation in cross-section of the capillary bed. This may be dependent upon proportion of open capillaries or upon the mean diameter of open capillaries. The latter possibility is suggested by the observations of Hartman, Evans and Walker (1929–30) that capillaries in the sartorius of the living cat vary in diameter but do not commonly close entirely. Since our subjects were at rest the capillary circulation in skeletal muscles does not appear to depend in any simple manner on rate of carbon dioxide production but must be under nervous or hormone control.

SUMMARY

Blood in the femoral vein of man represents a considerable fraction of the skeletal muscles. Its oxygen saturation in 10 men with normal circulation ranged from 56.6 to 90.2 per cent when the subjects were reclining and from 30.8 to 65.4 per cent when they were erect. On the average, the volume flow of blood through the legs was diminished one-half by this change in posture. The wide range of values found for oxygen saturation in different individuals indicates that in rest the cross-sectional area of the capillary bed can be varied more or less independently of carbon dioxide production.

BIBLIOGRAPHY

BENEDICT, F. G. AND C. G. BENEDICT. 1924. Proc. National Acad. Sci., x, 498.
BOCK, A. V., D. B. DILL AND H. T. EDWARDS. 1930. Journ. Clin. Inv., in press.
BLALOCK, A. 1929. Arch. Surg., xix, Pt. 1, 898.
GROLLMAN, A. 1929. This Journal, lxxxix, 584.
HARTMAN, F. A., J. I. EVANS AND H. G. WALKER. 1929. This Journal, xc, 668.
THOMPSON, W. O., P. K. THOMPSON AND M. E. DAILEY. 1928. Journ. Clin. Inv., v, 573.

# OBSERVATIONS ON HOLDING THE BREATH

EDWARD C. SCHNEIDER

*From the Wesleyan University, Middletown, Conn.*

Received for publication March 31, 1930

The ability to hold the breath varies with the individual. Whether this is determined more by physiological or psychological factors is as yet an unsettled question. Some of our experiences with breath holding shed light on this problem but do not clearly show to what extent the personal equation is involved.

During the latter part of the year 1918 and early in 1919 we secured data from 318 American aviators in France who held the breath for from 29 to 128 seconds. The mean time of the hold was 67.8 ± 1.2 seconds. These men were instructed to expire once as deeply as possible, then inspire fully and hold the breath as long as possible. That this great difference in the ability of individuals to hold the breath may have been determined by both physiological and psychological factors is indicated by the time required to render men semiconscious or unconscious by the breathing of pure nitrogen. In two investigations (Lutz and Schneider, 1919; Schneider and Truesdell, 1923) men, while breathing from a Larson automatic respirometer, were suddenly, but without knowing when, given pure nitrogen. In these experiments it was necessary for the observer, who was responsible for the welfare of the subject, to be familiar with the objective symptoms of anoxemia. When unconsciousness is impending (Schneider, 1924) the facial expression tends to become mask-like, the eyes to converge, and the pupils to dilate. In something over 200 tests on 45 men the length of the period of nitrogen breathing ranged from 47 to 112 seconds, with a mean time of 82 seconds. This range of upper and lower limits for nitrogen breathing indicates that men who fail to hold the breath for less than 30 or 45 seconds do so because they are not willing, or are mentally and nervously unable, to withstand the symptoms induced. The upper limits of breath-holding are only reached by men who are able to withstand some of the discomforts involved. It is unlikely that such men experience the degree of anoxemia that is obtained with nitrogen breathing.

The sensations experienced during an acute anoxemia, such as result from nitrogen breathing, are not distinctly unpleasant; in fact they are quite like those of nitrous-oxide anesthesia. With breath holding, however, there are, in addition to the dizziness and blurred vision of nitrogen

breathing, sometimes headache, profuse sweating, suffusion of the blood vessels of the head, pressure in the head, and a great sense of effort. When asked the reason for giving up the subject replies "I had to give up," "I felt I would burst," "the blood rushed to my head," or "things became blurred." It takes will power to withstand such sensations. Some men can hold the breath for only a few seconds after the uncomfortable sensations begin, while others hold until the discomfort is very pronounced.

One of the regular exercises in an undergraduate course in physiology includes a series of five experiments on holding the breath. In this series the man holds his breath until the breaking point is reached 1, after a moderate inspiration; 2, after a very deep inspiration; 3, after doing forced breathing for two minutes; 4, after three deep breaths of pure oxygen, and 5, after a period of forced breathing and three deep breaths of oxygen. In a group of 20 men, who really coöperated heartily, the range of time for the period of holding the breath in these five experiments was in 1, 30 to

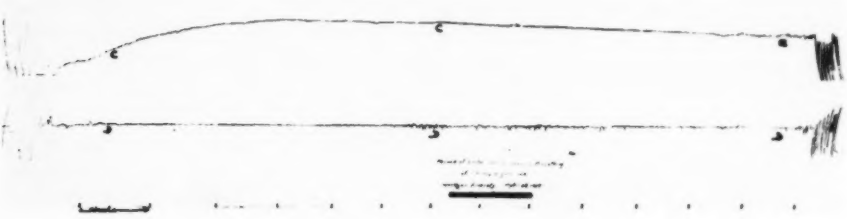


Fig. 1. Kymograph record of respiratory movements of E. L. Gaylor. C, costal movements. D, diaphragm or abdominal movements. Time record in minutes. Breath held 14 minutes and 2 seconds.

105 seconds; 2, 45 to 123 seconds; 3, 65 seconds to 4 minutes and 20 seconds; 4, 2 minutes and 10 seconds to 5 minutes and 40 seconds, and 5, 3 minutes and 30 seconds to 14 minutes and 2 seconds. On one occasion, by the fifth method, another man was recorded at 15 minutes and 13 seconds. The ability to hold the breath in the above group varied about as much as it did in the group of aviators, some of whom were believed to have coöperated with indifference. Thus in experiments 1 and 2 the range for this group of 20 men was between 30 and 123 seconds, while for the aviators it was between 29 and 128 seconds.

The fifth set of this series of experiments with the 20 men is of special interest because several of the holds are unusually long. The distribution of the holds was as follows: between 3 and 4 minutes, 1; 4–5 minutes, 3; 5–6 minutes, 2; 6–7 minutes, 3; 7–8 minutes, 1; 8–9 minutes, 4; 9–10 minutes, 1; 10–11 minutes, 3; 13–14 minutes, 1; and 14 to 15 minutes, 1. In this group of experiments the pulse frequency was counted, the arterial

blood pressures were determined, and a record of the respiratory movements was made on a kymograph by two Fitz pneumographs placed over the nipple line and the upper abdomen respectively. The pulse frequency and the systolic and diastolic arterial blood pressures invariably rose during the last part of the period of breath holding and sooner or later, usually well along toward the last part of the hold, the diaphragm contracted jerkily with greater and greater force.

The complete pulse and blood pressure data for the high record man, E. L. Gaylor, Jr., are given in figure 2 and the kymograph record of the respiratory movements is given in figure 1. Another subject, M. Knapp,

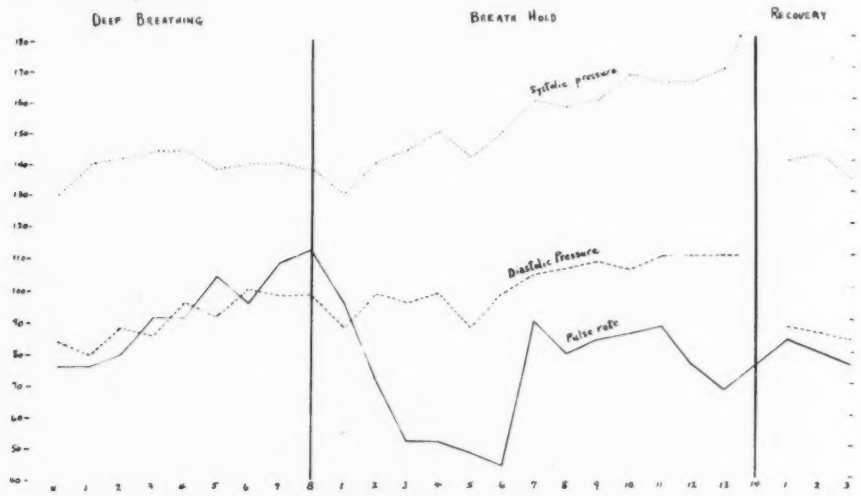



Fig. 2. Plotted circulatory data for Gaylor taken during a period of forced breathing and of breath holding. The line of abscissas gives time in minutes.

held his breath for 13 minutes and 34 seconds. Forced breathing accelerated the pulse and augmented the arterial blood pressure of both men. During the period when the breath was held the pulse frequency of each at first gradually fell below normal for from 5 to 6 minutes, after which it accelerated somewhat, but showed considerable variation during the last part of the hold. Both the systolic and diastolic arterial blood pressures showed a marked rise; the systolic pressure of Gaylor rose from 130 to 180 mm. and that of Knapp from 124 to 195 mm. The diastolic pressure of Gaylor rose from 88 to 110 mm. and that of Knapp from 90 to 140 mm.

The pulse and arterial blood pressure responses are similar to those which occur when the carbon dioxide content of inhaled air is gradually

increased (Schneider and Truesdell, 1922), and are quite unlike the effects of anoxemia produced by the rebreathing method (Schneider and Truesdell, 1921). This no doubt means that the objective and subjective symptoms that occur during the holding of the breath are caused more by the accumulation of carbon dioxide than by a lack of oxygen in the body. All of our subjects, however, when the fifth plan was used became quite cyanotic during the last minutes of the period of breath holding.

Another observation on the breaking point in breath-holding lends support to the belief that the accumultion of carbon dioxide in the body is the chief cause of the break. If, as has been claimed by Flack (1919), breath-holding shows how soon there is "oxygen want" in the body; then, when the breath is held in an atmosphere of reduced oxygen, the time of holding should be materially shortened. On several occasions the writer held his breath in a low pressure chamber at various low pressures that simulated high altitudes. On one occasion he, with two observers, was subjected to low pressures that corresponded in turn to altitudes of 10,000, 14,000 and 16,000 feet. Just prior to entering the low pressure chamber he held his breath after a deep inspiration for 120 seconds. At the simulated altitude of 10,000 feet he held his breath for 122 seconds; at 14,000 feet, for 128 seconds; and at 16,000 feet, for 120 seconds. Evidently his breaking point was not determined in these trials by a want of oxygen, as the oxygen supply was much reduced when he was under the several degrees of low barometric pressure. At sea level the partial pressure of oxygen in the atmospheric air is approximately 159 mm.; while at the simulated altitudes of 10,000, 14,000, and 16,000 feet the partial pressure of oxygen was 108, 93, and 86 mm. respectively.

It is practically impossible for a man at sea level to voluntarily hold his breath until he becomes unconscious, but under the lowered barometric pressure of the low pressure chamber it can quite easily be done. In the tests reported above, the writer held his breath until he was unconscious at the simulated altitudes of 14,000 and 16,000 feet. His two companions in the chamber found it necessary to slap him on the back in order to again start his breathing. Loss of consciousness came on so insidiously that such an outcome was wholly unexpected. The notes recorded by the two observers state that the unconscious subject was very cyanotic. Seldom does one see a marked degree of cyanosis in breath-holding tests at sea level.

Some observations made on Pike's Peak suggest that still other factors of blood chemistry than those of the carbon dioxide and oxygen content may influence the breath holding ability. During a sojourn of nine days on Pike's Peak, altitude 14,110 feet, barometric pressure 455 mm., two subjects, W. R. and E. C. S., showed a falling off in the breath-holding ability. In Colorado Springs, altitude 6000 feet, barometer 615 mm., W. R.

repeatedly held his breath, after a single deep inspiration, for from 63 to 75 seconds. The day after he arrived on Pike's Peak he held his breath for 61 seconds, the next day he could hold it for only 40 seconds, on the sixth day the time was reduced to 32 seconds, and on the last day to 30 seconds. In Colorado Springs E. C. S. always managed to hold his breath for from 60 to 73 seconds. During his stay on Pike's Peak his record is as follows: 2nd day, 62 seconds; 3rd day, 54 seconds; 4th day, 49 seconds; 5th day, 43 seconds; 6th day, 38 seconds; 8th day, 43 seconds; and 9th day, 34 seconds. A similar falling off in the ability to hold the breath has been reported by other students of Alpine physiology. The reason for this reduction has not been determined, but we surmise that it is associated with the decrease in the alkaline reserve of the blood. This assumption is supported by the fact that the alveolar carbon dioxide pressure of the lungs, which gives an index of the changes in the alkaline reserve of the blood, steadily falls off for a period of 10 days to two weeks during a sojourn on Pike's Peak (Douglas, Haldane, Henderson and Schneider, 1913).

*The correlation of breath-holding with other respiratory factors.* There were 127 men among the Army group of aviators who were given the Low Oxygen Altitude Classification examination and the English Fitness tests. From these data it has been possible to correlate the ability of holding the breath with the lowest percentage of oxygen that can be tolerated, with the rate the individual consumes oxygen when he is sitting at rest, with the vital capacity of the lungs, and with the force that can be exerted by the expiratory muscles. To evaluate these relationships the coefficient of correlation has been calculated. This measures the degree of scatter or of concentration of the data. The interpretation of the degree of correlation will be made on the following basis (Rugg, 1917): when the coefficient of correlation is less than 0.15 to 0.20, the correlation is regarded as negligible or indifferent; when it ranges from 0.20 to 0.35, as present but low; and when it ranges from 0.35 to 0.50 or 0.60, as markedly present.

The correlation between breath-holding and the rate at which the individual uses oxygen and also the lowest degree of "oxygen want" that he can endure was made possible by the official army altitude classification examination. In this the aviator is required to rebreathe 52 liters of air from which the carbon dioxide is removed by sodium hydroxide. The test is terminated when the aviator reaches the point of complete mental inefficiency with unconsciousness impending, or when the circulatory mechanism indicates that the man is about to faint.

The coefficient of correlation of breath-holding with the rate at which the individual absorbs oxygen was only $-0.27 \pm 0.06$, and with the lowest degree of "oxygen want" it was as small as $-0.18 \pm 0.06$. Such low coefficients as these indicate that oxygen is by no means the primary factor in bringing on the breaking-point in breath-holding. While a low de-

gree of correlation between oxygen and breath-holding was suggested by our observations in the low pressure chamber and on Pike's Peak, yet our class laboratory tests showed that a large supply of oxygen in the lungs ordinarily extends the time of the hold. In the five tests of breath-holding in the series used in the laboratory, the time of the hold is often longer when just oxygen is inhaled than when hyperventilation of the lungs is alone employed as a preliminary measure. Of the 20 cases reported above, only six failed to do as well with the oxygen alone as they did with hyperventilation alone.

The coefficient of correlation between the breath-holding ability and the vital capacity of the lungs for 127 cases was $0.24 \pm 0.06$. This shows a correlation between the two, but it is too low to make the vital capacity of the lungs a highly important factor in determining the length of time the breath can be held. Among these men the vital capacity averaged 4330 cc., the smallest was 3090 cc. and the largest 6620 cc. The two men who had the largest vital capacity managed to hold the breath only 55 and 69 seconds respectively. The man with the smallest vital capacity, 3090 cc., held his breath 74 seconds. The longest hold, 128 seconds, was made by one whose vital capacity was 4300 cc.

The coefficient of correlation between breath-holding and the expiratory force was $0.46 \pm 0.05$. For the determination of the expiratory force the subject was asked to steadily blow the column of mercury in a U-tube manometer as high as possible, and not to swing it up by the momentum of the mercury. This gives a measure of the power of the abdominal and other expiratory muscles. The mean expiratory force for the 127 cases was $119.98 \pm 1.87$ mm. Hg, the range was from 68 to 208 mm. The coefficient of correlation of 0.46 is fairly high and indicates that there is a very reliable correlation between breath-holding ability and the power of the expiratory muscles.

It is interesting to find that the coefficient of correlation between expiratory force and vital capacity for our group of cases was only $0.25 \pm 0.06$, which is a decidedly lesser degree of correlation than obtained between breath-holding and expiratory force. Expiratory force and breath-holding both involve the personal factor of leaving to the subject the decision of when to quit; while the physiological and anatomical factors involved in expiratory force and vital capacity are similar, they do not seem to possess the common psychological factor, will power or determination, involved in expiratory force and breath-holding. The high correlation in the one case and the lower in the other suggest that it is the psychological rather than the physiological factors that determine the outcome. This is in accord with White's (1920) experience with respiratory tests from which he concluded that they were tests of the stability of the nervous system, rather than of cardiac and pulmonary conditions; and with Birley's (1918)

experience with English aviators during the war when he found that breath-holding, expiratory force, and other simple respiratory tests afforded valuable information in assessing temperament and susceptibility to shock.

Taken in their entirety the observations here recorded indicate that for the majority of men the length of time the breath can be held is determined more by psychological than by physiological factors. When, however, the length of time is determined by physiological factors, it is the composition of the blood, its alkaline reserve and its content of carbon dioxide, which is of primary importance; while a lack of oxygen is only a secondary cause in determining the length of the hold.

## BIBLIOGRAPHY


BIRLEY. 1918. Report of the Air Medical Investigation Committee, London, no. 3, 25.

DOUGLAS, C. G., J. S. HALDANE, Y. HENDERSON AND E. C. SCHNEIDER. 1913. Phil. Trans. Roy. Soc. London, Ser. B, cciii, 185.

FLACK, M. 1919. Lancet, 1, 210.

LUTZ, B. R. AND E. C. SCHNEIDER. 1919. This Journal, l, 327.

RUGG, H. O. 1917. Statistical methods applied to education. New York, 256.

SCHNEIDER, E. C. AND D. TRUESDELL. 1921. This Journal, lv, 223.

1922. This Journal, lxiii, 155.

1923. This Journal, lxv, 379.

SCHNEIDER, E. C. 1924. Military Surgeon, March.

WHITE. 1920. Amer. Journ. Med. Sci., clix, 866.

# REFLEXES IN THE HIND LIMBS OF CATS AFTER TRANSECTION OF THE SPINAL CORD AT VARIOUS LEVELS

S. W. RANSON AND J. C. HINSEY

*From the Institute of Neurology, Northwestern University Medical School, Chicago*

Received for publication April 5, 1930

We have undertaken to determine how far the reflex arcs required for standing and walking are complete in the spinal cord. The investigation has been limited to the hind limbs because, as is well known, in a decapitate cat, even though the transection has been made at the lower border of the medulla oblongata and four segments above the origin of the roots of the brachial plexus, the activity of the fore limbs is profoundly depressed and it is extremely difficult to secure any responses whatever from them (Sherrington, 1910; Beritoff, 1922).

It is well known that rhythmic stepping movements alternating in the two hind limbs occur in the spinal animal (Sherrington, 1910, 1913b; Miller, 1923). But no one so far as we have been able to learn has transected the spinal cord at various levels with a view to ascertaining what was the lowest level of transection which allowed of rhythmic stepping movements in the hind limbs and whether this, when it occurred, was well developed and as fully coördinated as spinal stepping can be or whether after more anterior sections the stepping was more perfect, and what was the lowest possible section compatible with full and perfect spinal stepping.

The rationale of this approach to the problem of spinal reflex function becomes evident when one thinks of the longitudinal arrangement of the fibers in the white matter of the spinal cord. The various levels of the gray matter are linked one with another by longitudinal association fibers. Even a purely segmental reflex with afferent and efferent conductors in a single spinal nerve might be expected to be influenced by these long intraspinal association pathways. It seemed to us that one reflex might be more dependent than another on these longitudinal pathways and the superjacent segments. We have investigated this question with reference to the various ipsilateral and contralateral reflexes, the Stütz reflex, standing, and the rhythmic movements of progression.

It is known that spinal animals are not entirely lacking in that extensor tonus of the limbs which is essential for supporting the weight of the body in the standing posture. Apparently this tonus is more marked in the spinal dog than in the spinal cat. Sherrington (1910) states that he

decapitate cat cannot stand and that when it is placed in the erect posture it immediately sinks—the limbs have no power to oppose gravity. Nothing is said of the behavior in this respect of the chronic low spinal cat. But the dog becomes able to stand some weeks or months after transection of the spinal cord at the 10th thoracic segment. The hind legs are capable of maintaining the extended posture and supporting the weight of the posterior half of the body for minutes at a time.

Two factors are apparently responsible for this difference between decapitate cats and chronic spinal dogs. Time is udoubtedly important especially in the cat. We have repeatedly seen chronic spinal cats stand for more than a minute but the decapitate cats which we have studied were unable to bear the weight of the posterior part of the body on the hind legs unless the standing reflex was frequently reinforced by pinching the tail. It also seems probable that there is a marked difference in this respect between the cat and dog. The neural balance in the spinal dog seems to be more favorable for extensor reactions. We have only prepared one decapitate dog, but when this preparation was supported in a hammock with the legs hanging pendent, it resembled somewhat a decerebrate preparation. All four limbs were held fully extended and the hind quarters of the animal could readily be raised and supported for at least 30 seconds by upward pressure against the pads of the hind feet. In our four decapitate cats we never saw a comparable extensor tonus.

It is of significance also that Sherrington used spinal dogs for the demonstration of the extensor thrust (1906) and that for chronic spinal preparations he preferred dogs (1910). It is also to be noted that in the spinal dog, the knee jerk may be tonic (Sherrington, 1909). In such preparations it seems quite possible that crossed extensor reflexes may be much more easily obtained than in the cat.

Methods. Naturally in such an investigation as this the post-operative treatment of the animal is of the first importance. The cats eat meat readily but drink relatively little milk. To obviate this deficiency in fluid intake, we made a practice of giving 80 cc. of warm salt solution subcutaneously every other day. The bladder and rectum were evacuated daily by manual pressure over the abdomen.

The spinal transections were performed aseptically under deep ether anesthesia. About 1 cm. of the dura was exposed and opened. The spinal cord was cut across with a sharp knife at the lower border of the laminectomy opening and the cord in front of the section was raised out of the dural sac until the entire area of its cut surface could be easily seen. This left no possibility of doubt that the cord had been completely divided. After all hemorrhage had ceased, the opening in the muscles was closed with silk sutures in the lumbo-dorsal fascia and the margins of the skin were brought together with skin clips. In all of our animals, the wounds

healed without infection, excepting three cats (nos. 13, 15 and 18) in which the clips broke through the skin on the sixth or seventh day and caused an infection of the subcutaneous tissue. In all three of these the autopsy showed that the wound in the muscles had healed completely and aseptically. The infection developed at the end of the first week, was subcutaneous and strictly localized. As it did not appear to have affected the reflex activity of the animal the data from these experiments were included in table 1.

The cats were allowed to survive the operation for periods varying from 2 to 23 days. The cats which were killed within the first two or three days after the operation had recovered from a previous operation (section of the posterior part of the right lateral funiculus) and were sacrificed early so that ascending degeneration resulting from the spinal transection should not complicate the other investigation.

The observations were made from day to day with the cats supported in a hammock of muslin stretched across a frame and with four large openings through which the legs hung pendent (fig. 2). The stimuli by which the reflexes were elicited while the cat was supported in this way were for the most part natural ones, e.g., pinching of the toes, upward pressure of the hand against the pads of the toes, and pinching the tip of the tail or the skin over the back of the sacrum or in the region of the perineum. In 11 of the animals at the end of the last period of examination in the hammock, the tibial nerve was exposed at the inner side of the ankle, cut, and stimulated through shielded electrodes with the faradic current.

Observations were also made on acute spinal preparations. For this purpose 4 cats were decerebrated and then the spinal cord was cut at L 1, L 1, L 2, and L 3 respectively.

Kymograph records were made of the responses of the isolated gastrocnemius and tibialis anterior muscles in thirteen of the chronic spinal preparations. For this purpose, immediately after the last examination in the hammock, the cats were decerebrated by the anemic method of Pollock and Davis. Every one of these animals came through the decerebration in excellent shape and made good preparations for the graphic registration of the reflexes. External heat was applied when needed to keep the body temperature normal.

The right leg was rigidly fixed by a drill through the lower end of the femur and by a clamp on the middle portion of the femur and another on the tibia. The drill and clamps were securely fastened by bolts to the table. The leg was so securely fixed that only the movements of the two muscles which were being studied could be transmitted to the recording levers. The hamstring muscles and the insertion of the quadriceps were severed. The contractions of the gastrocnemius and anterior tibial muscles were recorded on the kymograph by means of approximately

TABLE 1

*Summary of observations on reflexes in intact hind legs of chronic spinal cats*

a = absent; t = trace; p = present; g = good. The figures preceding these letters indicate the number of days after operation when the particular observations were made.

| CAT NUMBER | LEVEL OF TRANSECTION | IPSILATERAL FLEXION | CONTRALATERAL FLEXION | EXTENSOR REBOUND | CROSSED EXTENSION | STEPPING | STÜTZ | RESISTANCE TO FLEXION OF KNEE | STANDING (FIGURES IN PARENTHESES = SECONDS) | POSTURE | KNEE JERK | REMARKS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 17 | L 6 | 1 a | 1 a | | 1 a | 1 a | 1 a | | | | 1 a | |
| | | 4 a | 4 a | | 4 a | 4 a | 4 a | | | | 4 a | |
| | | 7 a | 7 a | | 11 a | 11 a | | | | | | |
| 20 | L 6 | 2 a | 2 a | | 2 a | 2 a | 2 a | | 2 a | | 2 a | |
| | | 4 a | 4 a | | 4 a | 4 a | | | | | | |
| | | 8 a | 8 a | | 8 a | 8 a | | | | | | |
| | | 12 a | 12 a | | 12 a | 12 a | 12 a | | 12 a | | 12 a | |
| 25 | L 16 | 1 a | 1 a | | 1 a | 1 a | 1 a | 1 a | 1 a | 1 | 1 a | |
| | | 2 a | 2 a | | 2 a | 2 a | 2 a | | 2 a | 2 Flaccid | 2 a | |
| | | 7 a | 7 a | | 7 a | 7 a | 7 a | | 7 a | 7 | 7 a | |
| | | 16 a | 16 a | | 16 a | 16 a | 16 a | | 16 a | 16 | 16 a | |
| 13 | L 5 | 8 g | 8 t | | 8 a | 8 a | 8 p | | 8 a | | 8 g | Infection |
| | | 11 g | 11 a | | 11 a | 11 a | 11 p | | | | 11 g | |
| 26 | L 5 | 1 g | 1 a | | 1 a | 1 a | 1 t | | | | 1 t | |
| | | 5 g | 5 p | | 5 a | 5 a | 5 p | 5 t | 5 a | | 5 p | |
| | | 13 g | 13 p | | 13 a | 13 a | 13 p | 13 t | 13 a | | 13 p | |
| 11 | L 4 | 1 t | 1 t | | 1 a | 1 a | 1 t | | | | | |
| | | 4 g | 4 g | 4 g | 4 t | 4 a | 4 p | | | | 4 p | |
| | | 8 g | 8 t | | 8 g | 8 a | 8 p | | | | 8 p | |
| | | 9 p | 9 t | | 9 p | 9 a | 9 p | | | | 9 p | |
| 12 | L 4 | | | | | | | | | | | Clips out |
| 10 | L 3 | 1 g | 1 g | 1 g | 1 a | 1 a | 1 t | | | | 1 g | |
| | | 4 g | 4 g | 4 g | | | 4 g | | | Ext. on 8th day | 4 p | |
| | | 8 g | 8 g | | 8 a | | 8 g | | | | 8 g | |
| | | | | | 9 t | 9 a | | | | | | |
| | | | | | 12 t | 12 p | | | 12 g | | | |
| | | 18 g | 18 t | | 18 g | 18 a | 18 g | | 18(120) | | 18 p | |
| 6 | L 3 | 1 p | 1 a | | 1 a | 1 a | 1 p | | | | 1 g | |
| | | 2 p | 2 t | | 2 a | 2 a | 2 g | | | | | |

TABLE 1—*Continued*

| CAT NUMBER | LEVEL OF TRANSECTION | IPSILATERAL FLEXION | CONTRALATERAL FLEXION | EXTENSOR REBOUND | CROSSED EXTENSION | STEPPING | STÜTZ | RESISTANCE TO FLEXION OF KNEE | STANDING (FIGURES IN PARENTHESES = SECONDS) | POSTURE | KNEE JERK | REMARKS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | L 2 | 1 p | 1 a | | 1 a | 1 a | 1 p | | | | | |
| | | 2 g | 2 a | | 2 a | 2 a | 2 p | | | | | |
| 7 | L 2 | 1 g | | | 1 p | | 1 g | | | | | |
| | | 2 g | 2 p | | | 2 a | 2 p | | | | 2 p | |
| | | 3 g | | | 3 g | 3 p | 3 g | | | | | |
| 9 | L 2 | 1 g | 1 t | | 1 t | 1 a | 1 t | | | | 1 g | |
| | | 2 g | 2 g | 2 g | 2 a | 2 a | 2 t | | | | | |
| | | 4 g | 4 p | 4 g | 4 p | 4 a | 4 t | | | | 4 g | |
| 2 | L 1 | 1 g | 1 p | | 1 p | 1 a | 1 p | | | | | |
| | | | | | | 2 a | 2 p | | | | | |
| | | 3 p | | | | 3 a | | | | | | |
| 5 | L 1 | 1 p | | | 1 a | 1 a | 1 g | | | | 1 g | |
| | | 2 p | 2 a | | 2 a | 2 a | 2 p | | | | 2 g | |
| 8 | L 1 | 1 p | | | 1 t | 1 a | 1 t | | | | | |
| | | 2 p | 2 a | | 2 a | 2 a | 2 t | | | | 2 g | |
| | | 8 g | 8 p | | 8 a | | 8 t | | | | | |
| | | 9 g | 9 a | | 9 a | 9 a | 9 t | | | | | |
| 14 | L 1 | 2 p | 2 a | | 2 a | | 2 p | | | | 2 g | Diarrhea |
| 24 | L 1 | 1 g | 1 t | | 1 a | | 1 p | | | 1 Flex. | | |
| | | 3 g | 3 p | 3 p | 3 a | 3 p | 3 g | 3 p | | 3 Ext. | | |
| | | 7 p | 7 t | | 7 a | 7 t | | 7 t | 7(30) | 7 Ext. | 7 p | |
| | | 14 p | 14 a | 14 p | 14 a | 14 t | 14 g | 14 g | 14 a | 14 Ext. | | |
| 16 | T 13 | 4 p | 4 p | 4 p | 4 a | 4 a | 4 g | | | | 4 p | |
| | | 7 g | 7 a | | 7 p | | 7 g | | 7(20) | | 7 g | |
| | | 11 p | 11 t | 11 t | 11 a | 11 a | 11 p | | | | 11 p | |
| | | 14 p | 14 t | | 14 a | | | | | | | |
| 22 | T 13 | 2 g | 2 g | | 2 a | | | 2 t | 2(10) | 2 Flex. | 2 g | |
| | | 4 g | 4 g | 4 t | 4 a | 4 p | | 4 g | | 4 Ext. | 4 g | |
| | | 8 g | 8 g | 8 p | 8 t | | 8 g | 8 g | 8(20) | 8 Ext. | | |
| | | 22 g | 22 g | | 22 t | 22 p | 22 g | 22 p | 11(80) | 22 Ext. | | |

TABLE 1—*Concluded*

| CAT NUMBER | LEVEL OF TRANSECTION | IPSILATERAL FLEXION | CONTRALATERAL FLEXION | EXTENSOR REBOUND | CROSSED EXTENSION | STEPPING | STÜTZ | RESISTANCE TO FLEXION OF KNEE | STANDING (FIGURES IN PARENTHESES = SECONDS) | POSTURE | KNEE JERK | REMARKS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 3 | T 12 | 1 g | 1 p | | 1 a | 1 a | 1 g | | | | 1 g | |
| | | 2 g | 2 p | | | 2 t | 2 g | | | | | |
| 15 | T 12 | 5 g | 5 p | 5 g | 5 g | 5 a | 5 g | | | | 5 g | |
| | | 8 g | 8 g | 8 g | 8 p | 8 p | | | 8(15) | | | |
| | | 9 g | 9 g | | 9 p | 9 g | | | | 9 Ext. | | Infec- |
| | | 11 g | 11 g | | 11 p | 11 g | 11 g | | 11(20) | 11 Ext. | 11 p | tion |
| | | 12 g | 12 g | | 12 p | | | | | | | |
| 18 | T 10 | 1 g | | | 1 g | 1 p | 1 g | | | | 1 p | |
| | | 4 g | | | 4 p | 4 t | | | | | 4 p | Infec- |
| | | 6 g | 6 p | | 6 p | 6 a | 6 p | | | | 6 t | tion |
| 4 | T 8 | 1 g | 1 p | 1 p | | 1 g | 1 g | | | | 1 p | |
| | | 2 g | | | 2 a | 2 t | 2 t | | | | 2 p | |
| 19 | T 8 | 2 g | 2 p | 2 p | 2 a | 2 p | 2 g | 2 p | 2(30) | | 2 g | |
| | | 4 g | 4 p | 4 p | 4 a | | 4 g | | 4(65) | | 4 g | |
| | | 8 g | 8 t | 8 p | 8 t | 8 g | | 8 p | | | 8 g | |
| | | 12 g | 12 p | 12 p | 12 t | 12 g | | 12 p | 12(200) | | | |
| 27 | T 8 | 1 g | 1 a | | 1 a | 1 t | 5 g | 1 p | 1(25) | 5 Flac. | 1 p | |
| | | 5 g | 5 t | | | 5 g | | | 5(50) | 18— | 5 g | |
| | | 18 g | 18 a | | 18 a | 18 g | 18 g | 18 g | 18(165) | Ext., L. | 18 p | |
| | | | | | | | | | | Flex., R. | | |
| 21 | T 4 | 1 g | | | 1 p | 1 p | 1 p | | | | 1 p | |
| | | | | | 2 p | 2 g | 2 g | 2 a | 2(15) | 2 Flac. | | |
| | | 4 g | 4 t | | 4 a | 4 p | | 4 p | 4(15) | 4 Ext. | 4 p | |
| | | 8 g | 8 t | | 8 p | 8 t | 8 g | 8 p | | | | |
| | | | | | 23 p | 23 g | 23 g | 23 p | 23(105) | 23 Flac. | 23 g | |
| 23 | T 4 | 1 g | 1 a | | 1 a | 1 t | | 1 p | 1(45) | 1 Ext. | 1 g | |

isotonic levers with long rubber bands used in the place of weights. The gastrocnemius, although it is a potential flexor of the knee as well as an extensor of the ankle, always contracts in company with the other extensors and never takes part in flexor reflexes (Sherrington, 1913a).

In both legs the tibial nerve was exposed and divided at the medial side of the ankle. The nerves were stimulated with shielded platinum electrodes.

Sometimes later in the experiment the left femoral nerve was also exposed, divided and stimulated. For the purpose of comparison, similar records were made from decapitate and decerebrate preparations, and in these preparations, the saphenous nerve as well as the femoral and tibial was stimulated.

In the experiments on chronic spinal cats a Harvard coil was used with automatic interruptions at the rate of 50 per second and the break shocks were not short circuited. The primary current was approximately 2

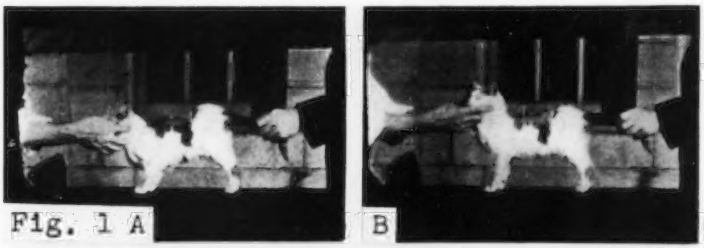


Fig. 1. Cinematographic pictures illustrating the ability of a chronic spinal cat to stand. The spinal cord had been cut through the last thoracic segment eleven days before the pictures were taken.

Fig. 2. Cinematographic pictures of the crossed flexion reflex in the chronic spinal cat eleven days after section of the spinal cord through the last thoracic segment. (A) Before the stimulus the left leg is held in a position of extension. (B) The left leg was flexed in response to pinching the toes of the right foot. (C) Extensor rebound following the crossed flexion reflex.

amperes. The position of the secondary coil varied from 13 to 6. The stimuli varied from those easily perceptible but not unpleasant on the tip of the tongue to strengths which were distinctly painful.

*The Stütz and stretch reflexes.* Pressure against the pads of the toes, simulating the pressure of the floor against the foot in the standing posture, causes a contraction of the muscles of the leg in such a way as to convert it into a prop or *Stütz*. Curiously enough this reflex escaped attention except in so far as the extensor thrust (Sherrington, 1906) may be regarded as one of its component parts, until Rademaker (1926) discovered it in his

decerebellate dogs where it is more pronounced than in normal animals. The reaction has been studied in detail by Schoen (1926) and by Pritchard (1926). They state that in thalamus animals, pressure on the sole of the foot causing extension of the toes and a dorsal flexion of the foot calls forth reflexly the extension and fixation of the entire leg so that it offers strong resistance to flexion at all joints. Flexor as well as extensor muscles participate in this reaction thus fixing the joints. In spinal animals, Schoen could not elicit the reaction. But he says there was a suggestion of it when the tonic neck reflex favored extensor tonus.

Magnus (1926) and Schoen (1926) distinguish between a positive and a negative Stütz reaction. The positive reflex was described in the preceding paragraph. The negative reflex is induced by passive plantar flexion of the toes. This causes a relaxation of extensor tonus and a decreased resistance to flexion of the entire leg. It can be demonstrated in the decerebrate animal even when the strong extensor tonus makes the demonstration of the positive reaction difficult or impossible. In the account which follows, when we speak of the Stütz reflex, we mean the positive reaction unless the negative is specified.

In producing the positive Stütz reaction, two kinds of stimuli are operative: 1, proprioceptive, from stretching of the plantar flexor muscles of the toes and the gastrocnemius, and 2, exteroceptive, from touching the pads of the foot (Magnus, 1926).

We have found what we take to be the Stütz reflex in spinal animals after the period of shock had passed. It is elicited by upward pressure against the pads of the foot but resistance to flexion disappears when the toes are put in the position of the negative Stütz reflex. So far as one can judge from the behavior of the intact limb the reaction is identical with that described by Schoen and Pritchard. To make the identification certain, kymograph tracings of the contractions of the individual muscles will be required.

A glance at table 1 will show that the reflex varied somewhat from day to day in the chronic spinal cats but that in general it was present or even well developed on the first day in those animals which were examined so soon after the operation. In some instances it has appeared as early as one and one-half hours after transection of the spinal cord.

The reaction could not be demonstrated at any time in the three cats in which the cord was cut at L 6, although these were observed for two weeks or more. The reflex was observed repeatedly in the two cats with transections at L 5. In those experiments in which the cut was made close to or through the lumbar enlargement, it was a matter of great importance to know just how far caudally the damage extended from the cut surface. Microscopical sections of these cords showed that following section at L 6, extensive anemic softening occurred in the cord behind the level of the transection.

The extensive lesions in the lumbrosacral cord in the three cats with sections at L 6 were evidently due to an interference with the circulation in the anterior spinal artery. The softening was for the most part confined to the area of distribution of the branches of this vessel. In order to determine whether anything similar occurred in the other spinal cords, sections were prepared from the two cords transected at L 5, one transected at L 4, one transected at L 1 and one transected at T 8. In each of these the segment through which the transection passed had been badly damaged but the next segment behind showed surprisingly little change, in hematoxylin and eosin or in cresyl violet preparations. The time which had elapsed was not sufficient for the formation of gitter cells in the degenerating fiber bundles. There was considerable destruction of nerve cells in the divided segment but practically none in the segment behind.

In two cats with transections at L 3 very good Stütz reactions were obtained. The quadriceps femoris muscle received its motor fibers from the fifth and sixth lumbar nerves with a small contribution from the fourth. It is therefore clear that for the occurrence of the Stütz reflex, no part of the cord above the level of the representation of the quadriceps is required.

Out of six decapitate cats, in which, after ligation of both carotid and both vertebral arteries, the spinal cord was cut across at its junction with the medulla oblongata and which then received 7 mgm. of ephedrine, the Stütz reaction was obtained in five. In one of these it was seen fifteen minutes after the transection. In none of the five was the reaction strongly developed, but in some of them it was possible to reinforce it by pinching the tail. Reinforced in this way, the Stütz reaction became so powerful that the hind legs would support the weight of the body for a time.

These tests were made as follows: The cats were supported in the hammock with the legs pendent. When an upward pressure was exerted by the hand against the pads of the toes there was slight but definite resistance to flexion. This resistance was usually increased if the pressure was made against the pads of both feet at the same time. Pressure of the hammock against the skin of the abdomen and groin serves as a stimulus reinforcing the Stütz reflex and the reflex is more easily demonstrated when the animal is in the hammock than when it is lying on its side.

Pinching the tip of the tail sometimes caused a very marked increase in the Stütz reaction so that the hind quarters of the animal could be elevated without bending the legs. When after some seconds the legs would begin to bend, pinching the tip of the tail would cause them to straighten out again. In this way, by frequently pinching the tail, the animal could be kept standing on the hand for two or three minutes at a time.

In many of the chronic spinal cats, a similar though usually less striking reinforcement of the reflex could be obtained by pinching the tip of the tail,

but this was not universally the case. In some of these cats, pinching the tail caused inhibition of the Stütz reflex. Inhibition of this reflex could always be produced by pinching the skin over the back of the sacrum or lower lumbar vertebrae, or even somewhat farther lateralward in the flank. Stimulation well to one side of the midline caused inhibition apparently equally well in both hind legs. No doubt this sensitive inhibitory reflex from the skin of the back is responsible for the difficulty in demonstrating Stütz-tonus when these spinal animals are lying on their backs.

The positive Stütz reflex was often unequally developed in the two legs and sometimes during one period of observation it would first be more marked on one side and then a few minutes later more marked on the other side. Again there would be periods when it would be increased or decreased on both sides together.

The negative Stütz reflex is more easily demonstrated in decerebrate than in spinal animals. But in the latter, upward pressure against the foot with the toes sharply plantar flexed, very seldom encountered any resistance. There was therefore a great difference in the resistance encountered in flexing the legs when the toes were extended in the position for the positive reaction and when they were flexed in the position for the negative reaction. In two of the experiments, some resistance was encountered even when the toes were plantar flexed. In these instances the negative Stütz reflex was imperfect and the positive reaction elicited from stretching the gastrocnemius and soleus was able to assert itself.

If the knee was first passively extended and then quite suddenly flexed by pressure exerted above the level of the ankle a very definite resistance to flexion was encountered. This reflex was noted as early as 24 hours after the operation in some of the experiments. In others it was not noted until the second, third, or even the fourth day. Observations in regard to this point were made in the last eight cats only, but the reflex was present in all except one of them and in that one the transection was at the level of the sixth lumbar segment. This resistance to flexion can most easily be explained as due to a shortening reaction (Sherrington, 1909) and stretch reflex (Liddell and Sherrington, 1924, 1925) in spite of the fact that it was observed within a day or two after the transection. Sherrington (1909) states that the shortest interval at which he found the shortening reaction in a spinal dog was 16 days, but on that occasion it disappeared again to return more permanently about a month after the transection.

When the Stütz reaction is tested in the intact leg it is impossible to say how much of the resistance to flexion is due to it and how much is due to the stretch reflex. The fact that there is a definite resistance to flexion of the knee when force is applied above the ankle clearly shows that the Stütz reaction is only one of the factors involved.

The Stütz reaction differs from the stretch reflex in three important

particulars (Schoen, 1926). It is partly of exteroceptive origin, while the stretch reflex is purely proprioceptive. Furthermore, stimuli restricted to the foot call forth contractions in the muscles of the entire leg, while, according to Sherrington, the stretch reflex is specific for the particular muscle in which the stimulus arises. In the third place, the Stütz reflex causes contraction in flexor as well as in extensor muscles while the stretch reflex has only been found in extensor muscles (Liddell and Sherrington, 1924, 1925; Schoen, 1926).

The extensor thrust was seen by Sherrington (1906) in the spinal dog. As we have already said, it seems probable that extension reflexes may be better developed in the spinal dog than in the spinal cat. We did not see an extensor thrust in our spinal cats but we did not look for it symetrically in all of them.

The knee jerk is also an ipsilateral extension reflex. It was present in all our cats with transections at L 5 or above and was seen from the first day after the operation as long as the observations were continued.

*Standing.* In our six decapitate cats, the fore legs remained flaccid throughout the experiment and the hind legs never developed sufficient tonus to enable them to support the weight of the hind quarters unless the Stütz reflex was reinforced by pinching the tail.

In the series of chronic spinal cats no attention was paid at first to the question of standing. When our attention had been attracted by the strong Stütz reflex to the possibility of standing, fourteen out of the twenty-three cats had already been killed. The remaining cats were each tested for standing on at least two different days and the results have been summarized in table 1.

The lowest level of transection after which we were able to make the hind legs bear the weight of the body in the standing posture was at the third lumbar segment. On the 18th day a cat (no. 10) with transection at this level, stood, bearing the weight of the posterior half of the body on the hind legs, for two minutes. It could not balance itself but it could support its own weight. The two cats in which the section passed through the fifth lumbar segment were unable to stand, although in both of them there was a positive Stütz reflex. Moorhouse (1930) has recently published graphic records of standing in spinal dogs.

*Posture.* Systematic observations on the posture of the hind legs were not begun until near the end of the series of experiments when only 8 cats remained. As might be expected after transection of the spinal cord through the sixth lumbar segment the legs hung flaccid when the cat was supported in the frame. But the remaining seven cats, with sections at or above L 1, all exhibited on one examination or another a definitely extended posture, i.e., the hind legs hung in a more extended position than they would if completely paralyzed. They therefore showed some extensor

tonus, though this extended posture was not nearly so great as that seen in decerebrate rigidity.

It should be noted that this extended posture was observed only when the cats were suspended in the hammock with legs hanging pendent. We have shown that thalamus cats, which, when on their feet crouch in a normal posture with a normal balance of tonus between flexors and extensors, exhibit very definite decerebrate rigidity when suspended in the same manner in the frame (Hinsey, Ranson and McNattin 1930). Unpublished experiments show that the extensor rigidity resulting from section of the posterior part of the lateral funiculus at the lower end of the cat's medulla oblongata can best be demonstrated when the animal is supported in this way. It seems that there may be something about this method of support that is favorable for the development of extensor tonus. The head is in the position of nearly minimal vestibular tonus (Magnus, 1924) and in any case vestibular tonus could not be a factor in spinal animals. We have repeatedly observed that tactile stimulation of the skin of the abdominal wall and inguinal fold causes a reinforcement of extensor tonus in these animals. We believe that the pressure of the hammock against the skin of the abdomen and groin serves as a stimulus which may at least partly account for the increased extensor tonus exhibited by thalamic and spinal animals when supported in this way (Hinsey, Ranson and McNattin 1930).

*Flexion and extension reflexes.* There is an enormous literature on the reflexes in spinal, decapitate, decerebrate and thalamic animals and even a cursory review of this work would exceed the limits of this paper. The observations which we have to report fall in line perfectly with those reported by Graham Brown (1911–1912) and support his conclusion that a flexion-producing stimulus does not merely activate the flexor and inhibit the extensor apparatus, but that it causes inhibition of the flexor and activation of the extensor apparatus as well, although these secondary effects may be neutralized and masked by its primary action. And in a converse manner the same holds true for an extension-producing stimulus. He shows how this leads to a variability of reaction. Although flexion is the usual response to ipsilateral stimuli, extension may at times be the result. In the same way a contralateral stimulus may call forth flexion instead of extension. There thus exists a "dilemma of reaction" which is very well illustrated by our results. We cannot go into the theoretical bearing of these results at this time but hope to do so in another paper. In this place we are concerned only with recording the results which we obtained and showing how the reflexes varied with varying levels of transection.

We have observed the flexion and extension reflexes and rhythmic stepping movements in spinal animals with intact hind legs at various

periods after the operation. For this purpose the animal was supported in a hammock with the legs hanging pendent. Rhythmic stepping movements could sometimes be elicited by pinching the tail or the skin of the perineum. Touching the foot and pinching the toes were the stimuli used in eliciting the flexion and extension reflexes. In some of the animals, at the termination of the last examination, the tibial nerve of the left leg was exposed and stimulated with shielded electrodes. The animals were then decerebrated and prepared for the registration of the reflex responses of the right gastrocnemius and tibialis anterior elicited by stimulation of the right and left tibial nerve at the inner side of the ankle. The fact that the reflexes observed in the intact legs showed so far as these could be observed the same character and variability as those recorded on the kymograph gives us added confidence in the results. We could usually predict the character of the tracings, which we would succeed in recording from the character of the reflex responses observed in the intact limb. In one instance, however, a cat which showed a well defined crossed extension reflex in the intact legs gave flexion as the only crossed response that could be recorded on the kymograph. In this instance a definite reversal of the type of reflex response was brought about by the procedures necessary for making the tracings, i.e., preliminary anesthesia, decerebration and the dissection of the legs.

It is well known that ipsilateral flexion is the easiest reflex to elicit in spinal animals. In fact, in the isolated spinal cord the neural balance seems to be definitely shifted toward the flexor side (Graham Brown, 1912; Forbes, 1922; Forbes and Baird, 1929; Forbes, Cobb and Cattell, 1923; Sherrington and Sowton, 1914). This shifting of the neural balance in spinal as compared with decerebrate preparations is largely to be explained by the cutting off of the impulses which normally play upon the extensor arcs of the spinal cord from the tonic center in the hind brain. It accounts for the ease with which contralateral flexion is obtained in spinal cats and the difficulty of obtaining ipsilateral extension under these conditions.

*Flexion reflexes.* Brisk ipsilateral flexion could be elicited in the hind legs by pinching the toes within fifteen minutes after decapitation. We have not examined the reflexes during the first few minutes following spinal transection but we have repeatedly obtained the reflex within an hour or two following the operation. It was the least variable of all the reflexes studied as can be seen by reference to table 1. It was present in every animal with the section at L 5 or above and although it varied somewhat in briskness and in the height to which the foot was withdrawn, it was always easily demonstrated. Cat 12 constitutes the only exception to this statement. On recovering from the anesthetic it exhibited signs of pain and tore out the skin clips. On the following day it was apathetic and the hind limbs entirely reactionless. On autopsy the cord was found to be soft.

In none of the three cats with sections through the sixth lumbar segment was it possible to demonstrate this or any other reflex in the intact limbs. The cats were kept 7, 12, and 16 days respectively and were in excellent condition. On autopsy the cord below the section appeared normal. But microscopic sections showed that extensive softening had occurred in the gray matter for at least two segments below the level of the transection. This disintegration of the sixth and seventh lumbar and first sacral segment from which the flexor muscles receive their innervation accounts for the complete absence of the flexion reflex in these three cats.

While the ipsilateral flexion reflex was present after transections through or above L 5, the movement was much brisker and more extensive after the higher transections especially those near the middle of the thoracic cord.

It has quite generally been accepted as a law that when a reflex response is elicited in one leg by stimulation of a sensory nerve of the opposite leg it involves the extensor muscles while the flexor muscles either show no change or undergo relaxation. But it is known that this law is subject to exceptions (Brown and Sherrington, 1912).

Contralateral flexion in the intact leg can be demonstrated when the cat is supported in the hammock with the legs hanging pendent. The most effective stimulus is the pinching of the toes together, the toes on each side of the foot being forced together on the dorsal side of the two middle toes. The pressure must be strong, uniform, and not too brief. When the reflex is present, the ankle slowly flexes and if the reflex is well developed, the knee and hip participate. Flexion persists while the stimulus continues but as soon as this ceases there usually occurs an extensor rebound which carries the leg into full extension. After a brief period of extension the leg returns again to a resting posture. In figure 2 there is represented the posture of the leg before the reflex begins (A), at the height of the response (B), and during the subsequent phase of full extension (C).

The rebound contraction of the extensor muscles can easily be mistaken for a primary crossed extension reflex. It may appear while the stimulus is being applied if the pressure on the toes is not maintained in a uniform manner.

As will be seen from table 1, contralateral flexion was demonstrated in the intact legs of most of the cats with transections at L 5 or higher. But it is more difficult to elicit than ipsilateral flexion and causes less movement of the leg and the movement is much slower. While contralateral flexion was more difficult to elicit than ipsilateral flexion, it was more often obtained than contralateral extension. When no clear-cut crossed reflex can be demonstrated it is often possible to demonstrate a decrease in the positive Stütz reaction, i.e., an inhibition of the extensor muscles as a result of contralateral stimulation.

It was sometimes noted that the character of the crossed reflex obtained depended upon the position in which the leg was hanging. In cat 7, when the right leg was hanging in an extended position, pinching of the toes on the left foot caused the right ankle to flex. But if the right leg were first flexed, the same stimulation would cause it to extend. Reversal of the type of reflex caused by changing the posture of the leg has been described in detail by Magnus (1924) under the designation of "Reflexumkehr."

Flexion in one intact leg can be elicited by electrical stimulation of the contralateral tibial nerve at the ankle. Tests of this sort were made on seven of the cats. The stimuli were rapidly repeated induction shocks perceptible but not at all painful on the tip of the tongue. In four of the cats the response was contralateral flexion chiefly at the ankle joint. This response was obtained from cat 18 with section at T 10, cat 19 with section at T 8, cat 22 with section at T 13, and cat 24 with section at L 1. Contralateral extension was seen in two of the seven cats, cat 21 with section at T 4 and cat 27 with section at T 8. In cat 16 with section at T 13, stimulation of the left tibial nerve caused brief extension followed by prolonged flexion in the right leg.

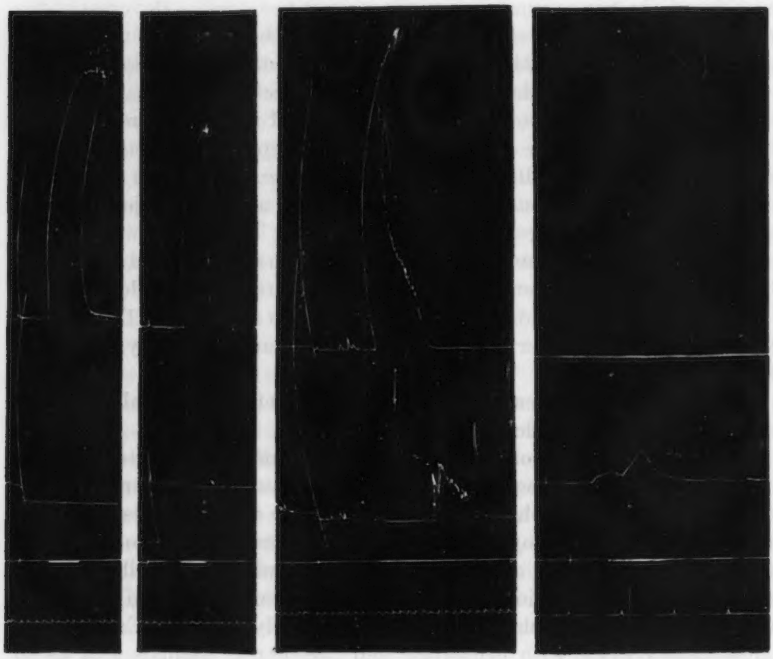
Tracings of the movements of the gastrocnemius and tibialis anterior muscles in the flexion reflex are shown in figure 3.

In this place we are concerned only with recording the results which we obtained and showing how the various reflexes varied with varying levels of transection. We wish, however, to emphasize certain points about the stimuli which we employed. The electrodes were applied to the tibial nerve at the inner side of the ankle. The stimuli were rapidly repeated make and break induction shocks (50 interruptions per second) and were well above the threshold though not excessively strong. Experiments subsequently made with decapitate and decerebrate animals lead us to believe that if we had used very weak or slowly repeated stimuli on the tibial nerve or had used the saphenous nerve instead of the tibial we might have obtained more orthodox results, that is to say, the crossed reflex might have been predominantly extensor instead of predominantly flexor in type.

In the contralateral flexion reflex (fig. 4) the tibialis anterior usually does not shorten as much as when it contracts in response to ipsilateral stimuli, but the contraction and relaxation may be equally abrupt. If the gastrocnemius exhibits tonic shortening at the time the stimulus is applied it may be inhibited and this muscle may lengthen (fig. 4). Rebound contractions of the gastrocnemius frequently occur after cessation of the stimulus, but they do not form a conspicuous feature of the tracings (fig. 5).

On the other hand, rebound contractions of the gastrocnemius after the cessation of the stimulus form a very prominent part of the response to

many ipsilateral stimuli. Occasionally the extensor contraction may be delayed for several seconds after the end of the stimulus and after the relaxation of the flexor contraction (fig. 7). In other cases it may follow




Figs. 3–6. Tracings recorded by tibialis anterior (above) and gastrocnemius (below). Time in seconds.

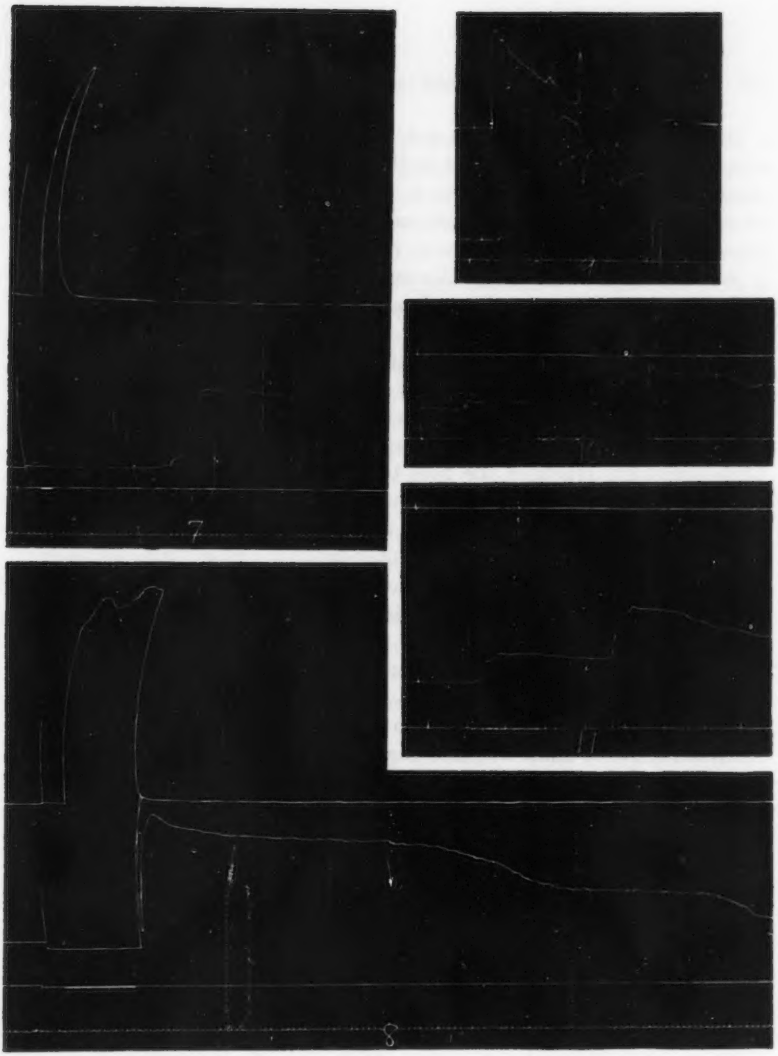
Fig. 3. Ipsilateral flexion reflex. Cat 22 with transection at T 13, 22 days after the operation. Tibial nerve, coil at,13 cm.

Fig. 4. Contralateral flexion reflex. Cat 22 with transection at T 13, 22 days after the operation. Tibial nerve—coil at 6 cm.

Fig. 5. Contralateral flexion reflex. Cat 21 with transection at T 4, 23 days after the operation. Tibial nerve—coil at 13 cm.

Fig. 6. Crossed extension reflex. Cat 24 with transection at L 1, 14 days after the operation. Tibial nerve—coil at 13 cm. and set at 45°.

immediately on the relaxation of the flexor contraction. The rebound contractions of the extensor muscle following the ipsilateral flexion reflex are usually very prolonged but occasionally this tonic contraction may be preceded by one or two quick contractions and relaxations (fig. 8).



Figs. 7–11. Tracings recorded by tibialis anterior (above) and gastrocnemius (below). Time in seconds.

Fig. 7. Ipsilateral flexion with delayed extensor rebound. Cat 22 with transection at T 13, 22 days after the operation. Tibial nerve—coil at 6 cm.

Fig. 8. Ipsilateral flexion followed by rhythmic extensor rebound and then by sustained extensor contraction. Cat 22 with section at T 13, 22 days after the operation. Tibial nerve—coil at 6 cm.

Fig. 9. Stepping movements superimposed upon a background of sustained contraction. Cat 15 with section at T 12, 12 days after the operation. Contralateral femoral nerve—coil at 6 cm.

Fig. 10. Crossed extension followed by postexcitatory extensor rebound. Cat 17 with section at L 6, 7 days after the operation. Tibial nerve—coil at 6 cm.

Fig. 11. Ipsilateral extension followed by postexcitatory extensor rebound. Cat 17 with section at L 6, 7 days after the operation. Tibial nerve—coil at 6 cm.

These rebound contractions are greater in extent, more frequent in occurrence and more prolonged in the records of the ipsilateral than in those of the contralateral flexion reflex. Yet when these reflexes are observed in the intact legs, rebound extension is seen quite regularly after contralateral flexion and very rarely after ipsilateral flexion. There is in this respect a marked difference in the behavior of the intact limb and that in which the muscles and nerves have been exposed in preparation for the tracings.

*Contralateral extension.* With the animal hanging in the hammock it was as a rule more difficult to elicit extension than flexion by stimulation of the opposite foot. It could more often be evoked with weak than with strong stimuli. Sometimes lightly touching the foot would cause extension while forcefully pinching the toes together would cause crossed flexion. One must, however, be continuously on guard against mistaking an extensor rebound which follows the cessation of a stimulus for a true crossed extension reflex which occurs during stimulation.

The reflex was most easily produced when the leg had previously been brought into a flexed position. This is another instance of reflex reversal called forth by changes in posture (Magnus, 1924). Sometimes crossed extension would follow the first contact of the hand with the foot but could not be elicited a second time.

Production of a crossed extension reflex on electrical stimulation of the tibial nerve at the ankle was also uncertain. As we have said, such stimulation caused in four out of seven cats flexion instead of extension.

When we succeeded in producing a crossed extension reflex, the contraction was not very high or prolonged (fig. 6). A study of the tracings shows that a pure crossed extension reflex unaccompanied by any contraction of the flexor muscle was obtained in 6 of the 13 cats. In four other cats, contractions of the gastrocnemius were obtained but they were complicated by flexor rebound or by alternate or cocontraction of the flexor muscle.

It might be expected that the level of the transection would be an important factor in determining whether the preparation would yield contralateral responses of the flexor or extensor type. But we could find no evidence that this was the case. It is true that no crossed extensor responses were obtained from either of the cats with transactions at L 5, but a fairly good contralateral contraction of the gastrocnemius (fig. 10) was obtained from cat 17 whose cord had been cut at L 6. This cord showed on microscopical examination extensive disintegration of the sixth and seventh lumbar and first sacral segments. In these segments the lateral funiculus, the lateral part of the anterior gray columns and parts of the posterior gray columns and posterior funiculi were largely intact. But extensive softening had occurred in the territory supplied by the anterior spinal artery, including the ventral funiculus and medial part of the

anterior gray columns. The commissures of the cord also seem to have been largely destroyed. In fact it is surprising that with the cord in such condition, any reflexes could be elicited at all. Yet a fairly good crossed response of the gastrocnemius was recorded in this cat and it was followed by a marked post-excitatory rebound (fig. 10). We also obtained in this cat an ipsilateral contraction of the gastrocnemius followed by post-excitatory rebound (fig. 11). The crossed response could be obtained only twice but the ipsilateral extension could be obtained as often as the stimulus was applied and always took the form shown in figure 11. It is therefore clear that surprisingly little of the cord needs to be intact for the occurrence of th· extensor reflexes. No response whatever could be obtained from the flexor muscles in this preparation. The gastrocnemius muscle must have received its innervation from the group of cells which persisted in the lateral part of the anterior horn. Probably the cells supplying the tibialis anterior were among those which had degenerated.

This cat with a badly damaged cord is the only one of the series which gave an ipsilateral extension reflex. Probably if we had used very weak stimuli we would have encountered this response more frequently.

The "dilemma of reaction" between flexion and extension in the contralateral response was just as evident after high as after low transections. Both types of responses were obtained after transections at T 4, T 8, T 10, T 12, T 13, L 1, L 2, L 3 and L 4.

*Extensor afterdischarge in spinal animals.* The extensor rebound following the ipsilateral flexion reflex gives evidence of very prolonged afterdischarge. The contraction illustrated in figure 8 lasted more than 2 minutes. Of course since this muscle was not deafferented it might be argued that this represented a shortening reaction superimposed upon the rebound contraction. But in the record reproduced in figure 7 the rebound contraction was delayed more than 20 seconds after the end of the stimulus. In this instance the inhibitory afterdischarge was unusually long and prevented the excitatory afterdischarge from making itself felt until after the lapse of many seconds. We have seen many of these delayed rebounds although usually the delay is not so long. It seems clear that in such reactions the excitatory afterdischarge must have persisted at least until the height of the rebound contraction was reached. Even allowing for the possibility of a shortening reaction being responsible for such long contractions as that shown in figure 8, there is evidence that extensor afterdischarge in spinal cats may last for many seconds. Inhibitory afterdischarge may last 4 seconds in spinal animals (Fulton, 1926, p. 346).

Two explanations have been advanced to account for the afterdischarge which is such a characteristic feature of reflex contractions. Sherrington (1925) has suggested that there may be a piling up of an excitatory sub-

stance in the neurones or at the synapses during stimulation and that this substance gradually dissipates itself in maintaining the discharge of the motor neurone after the stimulus has ceased. Another hypothesis has been advanced by Forbes (1922). He thought that a sensory impulse spreading out along branching pathways in the central nervous system would reach a given motor center as a succession of impulses, since each pathway would have a different conduction time. This would hold equally true for inhibitory and excitatory arcs. Fulton (1926) criticises this conception as follows: "Afterdischarge of 5 or 6 seconds' duration is sometimes observed in the crossed extensor response of the deafferented quadriceps and for impulses to be delayed to this extent at the rate of conduction in mammalian nerve, it would be necessary for the impulse to travel for a distance of 500 metres, i.e., for more than a quarter of a mile. Even with due consideration of the known complexity of central nervous paths and possible synaptic delays, this would almost amount to an impossibility."

Since prolonged afterdischarge occurs after low spinal transection in preparations in which the brain and the cervical and thoracic portions of the spinal cord can play no part it is obvious that any delay paths which function in these experiments must be restricted to the lumbosacral portion of the spinal cord. Such paths might be thought of as consisting of closed rings in the manner illustrated in figure 12. The diagram represents a longitudinal column of extensor motor neurones and associated premotor neurones arranged in such a manner as to account for the afterdischarge. It is known that in an extension reflex all of the extensor muscles contract and that in a flexion reflex all of the flexor muscles contract irrespective of what sensory nerve is used in calling forth the response. This could be brought about by the linking together of the motor or premotor neurones of similar function so that they would discharge simultaneously or in rapid succession. It is known that the motor impulses of an extensor afterdischarge are not sent out in simultaneous volleys, but are out of phase (Cooper and Adrian, 1924). An impulse transmitted to one of the premotor neurones in the diagram (fig. 12) would be propagated around the ring activating the motor neurones in turn. The motor impulses would therefore be out of phase. An impulse once started in the ring would go around and around until interrupted by synaptic fatigue, and as long as it continued to travel the circuit it would continue to activate the associated motor neurones. The diagram might be constructed many different ways and the one given in figure 12 is not intended as anything more than an illustration of the basic idea.

Since the afterdischarge seen in spinal animals with transections through the upper lumbar cord cannot be explained as due to impulses traveling delay pathways to the brain and since the length of such delay paths would have to be very great if they were to account for afterdischarges

lasting many seconds, it would seem that such pathways do not offer a satisfactory explanation of the phenomenon. Forbes (1929) has recently come to the conclusion that "the most prolonged afterdischarge of the crossed extension reflex would require a sort of central reverberation." The conception of reverberating neurone circuits has certain advantages. It does not require the postulation of anything different from or in addition to the conduction of the nerve impulse. If the circuit is long enough to prevent the quick onset of fatigue it accounts for afterdischarge lasting minutes at a time. In the particular form in which it is illustrated in figure 12, it would account for the linking together of the extensor motor neurones in such a manner that they would discharge regularly in rotation

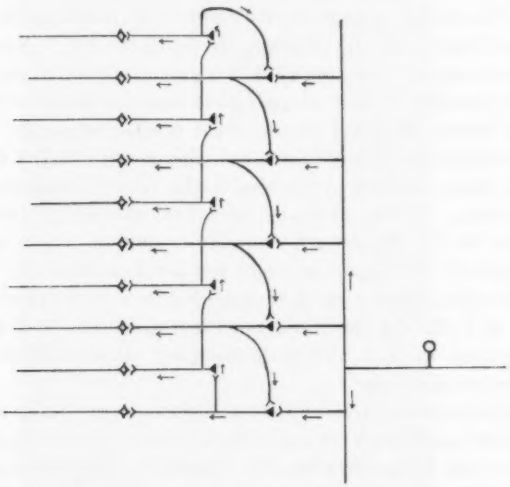


Fig. 12.—Diagram of a possible arrangement of motor and premotor neurones which would account for after discharge.

with relatively long periods of rest. This would account for the fact that in tonic contractions the motor impulses are out of phase (Cooper and Adrian, 1924), and are discharged from the motor neurones at very slow rates (Adrian and Bronk, 1929; Denny-Brown, 1929).

This scheme might well explain the primary and secondary waves in action potential records described by Cooper and Adrian (1924) in the crossed extension reflex during stimulation. The primary waves would correspond to synchronous discharges from the motor neurones due to sensory impulses acting on the premotor neurones shown in this diagram; while secondary waves would be due to the fact that the reverberation responsible for after discharge was brought into activity. If during stimu-

lation the afferent impulses were brought to the premotor neurones, one would expect a fairly synchronous discharge over the neurones of the final common path corresponding to stimulation frequency. At the cessation of stimulation, reverberation over the arcs shown in figure 12 would result in an asynchronous discharge of impulses which is characteristic of the afterdischarge, where, according to Cooper and Adrian (1924), "There is never any regular frequency in the electric responses or any indication of the rhythm which existed during the stimulation."

Diagrams of reverberating neurone circuits could be constructed to account for flexor afterdischarge and for inhibitory afterdischarge.

*Stepping.* All of our cats were carefully observed for the occurrence of stepping movements while supported in a hammock with the legs hanging pendent. This could be most easily elicited by pinching the skin of the perineum and less readily by pinching the tip of the tail. None of the cats showed "spontaneous" stepping while hanging in the hammock.

It was not possible to elicit stepping movements in any of the cats with transections below the level of the third lumbar segment. In the two cats with sections at L 5 and in one cat with section at L 4 which showed considerable reflex activity in the hind limbs, no rhythmic stepping movements were seen. In the cats with spinal transection at levels from the third lumbar to the thirteenth thoracic segments, reflex stepping was either very poorly developed or could not be elicited at all. Fairly good rhythmic stepping alternating in the two legs was seen in one cat with the transection at T 12. In the two cats with transections at T 8 and the two with transections at T 4, rhythmic stepping alternating in the two legs was much better developed.

The question arises as to how far the improvement in the stepping with sections farther and farther forward can be correlated with an improvement of the flexion and extension reflexes. There is a parallel improvement of the ipsilateral flexion reflex which is much more brisk and extensive after sections through the midthoracic than after sections through the lumbar segments of the cord. On the other hand, as is shown by table 1, there is no parallel improvement in the contralateral flexion or extension reflexes. The dilemma between these two responses persists after midthoracic as well as after lumbar sections. We are inclined to believe that the improvement in the ipsilateral flexion and reflex stepping at the higher levels is due to the fact that a larger number of longitudinally conducting pathways are intact.

In a few of the kymograph records we saw evidence of stepping, that is, of rhythmically repeated contraction and relaxation occurring reciprocally in the flexor and extensor muscles and of a rate definitely slower than that of the scratch reflex. Sometimes such movements occurred during or immediately after stimulation applied to the contralateral femoral or

tibial nerves. This was to be seen in the tracings made by six of the cats, but the stepping was very imperfect in four of these (sections at L 1, T 12, T 10, and T 8). In both cats with transections at T 4, excellent stepping was recorded in the tracings (fig. 13). In fact, this stepping was so perfect as to indicate that the controlling neural mechanism was quite complete. But the movements of progression are not "spontaneous" as they are in thalamic or after high decerebrations.

Fig. 13. Rhythmic stepping movements in tibialis anterior (above) and gastrocnemius (below). Cat 21 with section at T 4, 23 days after the operation. Contralateral femoral nerve—coil at 8 cm.

SUMMARY

In cats the spinal cord was transected at levels varying from L 6 to T 4. Varying amounts of time up to 23 days were allowed for recovery. Decapitate cats and acute spinal cats were also studied.

The animals were studied while supported in a hammock with legs hanging pendent. Later, kymograph records were made of the responses in the isolated gastrocnemius and tibialis anterior muscles to stimulation

of the ipsilateral and contralateral tibial nerves at the ankle with rapid faradic make and break shocks.

With the animal supported in the hammock there was considerable extensor tonus which showed itself best when pressure was made against the pads of the toes in the position of the positive Stütz reaction. This reaction was as well developed after transections at L 3 as after transections at higher levels. There was also some, though less marked, resistance to flexion of the knee when the force was applied above the ankle. The Stütz reflex as elicited from the intact leg cannot be sharply distinguished from the stretch reflex. It is inhibited by pinching the skin on the back of the sacrum and often reinforced by pinching the tip of the tail. Tactile stimuli to the skin of the abdominal wall and groin (pressure of the hammock) also serve to reinforce extensor tonus in the hind limbs.

Cats with transections at or above L 3 were able to bear the weight of the posterior part of the body on the hind legs for varying periods up to 3 minutes.

When supported in the hammock where extensor tonus was reinforced by pressure against the abdominal wall and groin the hind legs were often held in an extended posture, though sometimes they hung in the position of rest or were even slightly flexed.

The ipsilateral flexion reflex was obtained with great regularity in all animals with transection at L 5 or above.

The crossed response was more variable. Crossed flexion was more often obtained than crossed extension. Sometimes lightly touching a foot would cause crossed extension and forcefully pinching the toes together would cause crossed flexion. Electrical stimulation of the tibial nerve at the ankle more often caused crossed flexion than crossed extension both in the intact leg and when the contractions of the tibialis anterior and gastrocnemius were recorded on the kymograph. In the crossed response there is a dilemma of reaction which makes these reflexes uncertain and difficult to obtain. This dilemma is just as pronounced after high as after low transections.

The contraction of the tibialis anterior in response to ipsilateral stimulation of the tibial nerve is often folllowed by a prolonged rebound contraction of the gastrocnemius. The after discharge in this extensor rebound contraction may last for many seconds. A diagram of a reverberating neurone circuit is given which could be used to explain afterdischarge.

None of the cats showed "spontaneous" stepping but in many of them stepping could be elicited by pinching the skin of the perineum. Good rhythmic stepping movements alternating in the two legs were seen after transections at T 4 and T 8. After transections below the level of T 12 stepping movements could not be elicited or were poorly developed and difficult to obtain.

After the higher sections, stepping was more perfectly coördinated and movements were of wider excursion. In the intact limbs the ipsilateral flexion reflex was brisker and more extensive in the cats with high than in those with lower transections. It would seem reasonable to assume that the ascending and descending paths in the funiculus proprius of the cord and the gray matter in the upper lumbar and thoracic segments may take some part in the reflex responses of the hind limb and that the differences in stepping and in the ipsilateral flexion reflex after higher and lower transections may depend upon the extent to which these intraspinal conduction paths are left intact.

## BIBLIOGRAPHY


ADRIAN, E. D. AND D. W. BRONK. 1929. Journ. Physiol., lxvii, 119.

BERTIOFF, J. S. 1922. Pflüger's Arch., excix, 248.

BROWN, T. GRAHAM. 1911. Quart. Journ. Exper. Physiol., iv, 331.

1912. Quart. Journ. Exper. Physiol., v, 237.

BROWN, T. GRAHAM AND C. S. SHERRINGTON. 1912. Journ. Physiol., xliv, 125.

COOPER, S. AND E. D. ADRIAN. 1924. Proc. Roy. Soc., Series B, xcvi, 243.

DENNY-BROWN, D. 1929. Proc. Roy. Soc., Series B, civ, 252.

FORBES, A. 1922. Physiol. Reviews, ii, 361.

1929. The mechanism of reaction. Chap. 3, p. 152. The foundations of experimental psychology. Clark University Press, Worcester, Mass.

FORBES, A. AND P. C. BAIRD, JR. 1929. This Journal, lxxxvii, 527.

FORBES, A., S. COBB AND H. CATTELL. 1923. This Journal, lxv, 30.

FULTON, J. F. 1926. Muscular contraction and reflex control of movement. Baltimore.

HINSEY, J. C., S. W. RANSON AND R. F. MCNATTIN. 1930. Arch. Neurol. and Psych., xxiii, 1.

LIDDELL, E. G. T. AND C. S. SHERRINGTON. 1924. Proc. Roy Soc., Series B, xcvi, 212.

1925. Proc. Roy. Soc., Series B, xcvii, 267.

MAGNUS, R. 1924. Körperstellung. Berlin.

1926. Deutsch. Zeitschr. f. Nervenheilk., xciv, 141.

MILLER, F. R. 1923. Trans. Roy. Soc. Canada, 3rd series, xvii, Sec. v, 29.

MOORHOUSE, V. H. K. 1930. This Journal, xcii, 716.

PRITCHARD, E. A. B. 1926. Pflüger's Arch., ccxiv, 148.

RADEMAKER, G. G. J. 1926. Deutsch. Zeitschr. f. Nervenheilk., xciv, 144.

SCHOEN, R. 1926. Pflüger's Arch., ccxiv, 21, 48.

SHERRINGTON, C. S. 1906. The integrative action of the nervous system. New Haven.

1909. Quart. Journ. Exper. Physiol., ii, 109.

1910. Journ. Physiol., xl, 28.

1913a. Quart. Journ. Exper. Physiol., vi, 251.

1913b. Journ. Physiol., xlvii, 196.

1925. Proc. Roy. Soc., Series B, xcvii, 519.

SHERRINGTON, C. S. AND S. C. M. SOWTON. 1914. Journ. Physiol., xlix, 331.

THE AMERICAN JOURNAL OF PHYSIOLOGY is issued monthly by the American Physiological Society under the direction of the Council of the Society. From three to four volumes, each of about seven hundred and twenty pages are published yearly. The subscription price per volume in the United States and Canada is $7.50; in other countries, $8.00.

---

Manuscripts submitted for publication may be sent to any member of the Council or to the Managing Editor, Dr. D. R. Hooker, 19 W. Chase Street, Baltimore, Md. They should be packed flat, carefully protected from injury, and insured.

Each article should conclude with a brief summary of results suited to the needs of abstract journals.

References to literature should accord with the following arrangement. In the text author's name is to be followed by the year date of the publication in parenthesis, thus Smith (1911). If the author's name is not mentioned in the text, the parenthesis is to contain that name as well as the date, thus (Smith, 1911). If reference is made to more than one publication of an author in a given year, the year date is to be followed by the letters a, b, c, etc., thus Smith (1911a). The bibliography at the end of the article is to list all text references according to authors' names, with initials, alphabetically arranged, thus, Smith, J. C. 1911. Journ. Biol. Chem., xii, 306.

Authors are especially urged to verify personally the accuracy of their bibliographies by reference to the original sources, since inadvertent errors cause readers great inconvenience.

All figures for illustration must be submitted in such form as to admit of photographic reproduction without retouching or redrawing. Marginal letters or figures cannot be set in type and must, therefore, be drawn in India ink by the author and regarded as part of the figure. Unless specific instructions are given by authors, the printer will be allowed to use his judgment in determining the amount of reduction on all figures. The JOURNAL cannot accept illustrations which, when reproduced for printing, exceed the size of a JOURNAL page (4½ × 7½ inches), unless the author is prepared to pay the cost of an insert page.

Plotted curves must be drawn in India ink on blue lined coördinate paper. Blue ink will not show in reproduction, so that all guide lines, letters, etc., must be in India ink.

Best results are obtained when such figures are made proportional to the shape of the JOURNAL page so that, when reduced, they will fit without undue loss of space.

---


# AT THE MENOPAUSE

The distressing symptoms that so often accompany the climacteric, but more particularly the aggravated symptoms of the artificial menopause, are frequently controlled by the administration of

LUTEIN TABLETS, H. W. & D.
LUTEIN SOLUTION AMPULES, H. W. & D.

The choice of the medication depends, of course, on the judgment of the physician as to whether oral or hypodermatic administration is indicated. Both products represent the

## CORPUS LUTEUM OF THE SOW

Lutein Tablets, H. W. & D., are unmodified by treatment with solvents or by exposure to temperatures above animal body heat in the drying process. All separation of extraneous matter is made by mechanical means and all drying is *in vacuo*. The unaltered *corpus luteum* should, therefore, be presented in our products and clinical experience with them should demonstrate their therapeutic activity.

Ovarian dysfunction as evidenced in dysmenorrhea and amenorrhea is also an indication for Lutein medication. Where the diagnosis of such dysfunction was well established, definite therapeutic results have been reported.

WHOLE OVARY TABLETS, H. W. & D.
OVARIAN RESIDUE TABLETS, H. W. & D.

are also offered for those who prefer, for certain indications, the use of the whole gland or of the residue remaining after corpus luteum separation.

*Literature furnished on request*

H. W. & D.—SPECIFY—H. W. & D.—SPECIFY—H. W. & D.—SPECIFY—H. W. & D.

**HYNSON, WESTCOTT & DUNNING : : Baltimore, Md.**

# INDEX

## AMERICAN JOURNAL OF PHYSIOLOGY

An Index to Volumes 61 to 90 inclusive of the American Journal of Physiology will be published about December, 1930, the price of which will be $4.00 per copy if ordered in advance.

A few copies of the Index to Volumes 1 to 30 inclusive (price $4.00) and of the Index to Volumes 31 to 60 inclusive (price $2.50) are still available.

Orders should be sent to the American Journal of Physiology, 19 W. Chase Street, Baltimore, Md.

# PHYSIOLOGICAL REVIEWS

### Contents of Volume VIII, 1928


KENNETH J. FRANKLIN: Physiology and pharmacology of veins

S. A. ASDELL: The growth and function of the corpus luteum

HENRY LAURENS: Physiological responses to light

O. R. LANGWORTHY: The control of posture by the central nervous system

S. L. WARREN: Physiological action of x-rays

H. M. VERNON: Industrial fatigue

FLORENCE R. SABIN: Bone marrow

L. N. KATZ: Significance of the T wave of the electrocardiogram

H. B. VICKERY AND T. B. OSBORNE: Protein molecule

C. P. STEWART AND G. H. PERCIVAL: Calcium metabolism

B. P. BABKIN: Gastric digestion

HENRI FREDERICQ: Chronaxie

MRS. EDWARD MELLANBY: The influence of diet on tooth formation

H. S. RAPER: The aerobic oxydases


### Tentative Contents of Volume X, 1930


A. E. MIRSKY: The Hemoglobin Compounds, with Special Reference to Protein Chemistry and the Significance of the Heme Pigments in Tissue Oxidations

H. M. EVANS: The Anterior Hypophysis

J. H. BURN: The Errors of Biological Assay

T. GRAHAM BROWN: The Technique for Recording Reflexes from the Decerebrate Preparation

H. E. MAGEE: The Physiology of the Small Intestines

E. D. ADRIAN: The Action of the Sense Organs

LEO LOEB: Individuality and Transplantation

V. E. HENDERSON: The Present Status of the Narcosis Theories

W. F. VON OETTINGEN: Excretion and Distribution of Bismuth Compounds

S. W. BRITTON: Adrenal Insufficiency

BALDUIN LUCKÉ: Cellular Osmosis

DAVID RAPPORT: The Interconversion of Foodstuffs

A. B. HASTINGS: Comparative Effects of Chlorides, Bromides and Iodides on Animals

CARL F. CORI: Carbohydrate Metabolism


---

Subscriptions may be sent to D. R. Hooker, Managing Editor, 19 W. Chase Street, Baltimore, Md. $6.00 per volume, domestic. $6.50 per volume, foreign.